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(71) Applicant (for all designated States except US): INFECTIO DIAGNOSTIC (I.D.I.) INC. [CA/CA]; 4ème étage, 2050, boulevard René Lévesque Ouest, Sainte-Foy, Québec GIV

(72) Inventors; and

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2K8 (CA).

(75) Inventors/Applicants (for US only): BERGERON, Michel, G. [CA/CA]; 2069, rue Brûlard, Sillery, Québec G1T 1G2 (CA). PICARD, François, J. [CA/CA]; 1245, rue de la Sapinière, Cap-Rouge, Québec G1Y 1A1 (CA). OUELLETTE, Marc [CA/CA]; 1035 de Ploermel, Sillery, Québec G1S 3S1 (CA). ROY, Paul, H. [US/US]; 28, rue Charles Garnier, Loretteville, Québec G2A 2X8 (CA).

(74) Agents: DUBUC, Jean, H. et al.; Goudreau Gage Dubuc & Martineau Walker, The Stock Exchange Tower, Suite 3400, 800 Place Victoria, P.O. Box 242, Montreal, Québec H4Z 1E9 (CA).

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#### (57) Abstract

DNA-based methods employing amplification primers or probes for detecting, identifying, and quantifying in a test sample DNA from (i) any bacterium, (ii) the species Streptococcus agalactiae, Staphylococcus saprophyticus, Enterococcus faecium, Neisseria meningitidis, Listeria monocytogenes and Candida albicans, and (iii) any species of the genera Streptococcus, Staphylococcus, Enterococcus, Neisseria and Candida are disclosed. DNA-based methods employing amplification primers or probes for detecting, identifying, and quantifying in a test sample antibiotic resistance genes selected from the group consisting of blatem, blazob, blasho, blazom, blaZ, aadB, aacC1, aacC2, aacC3, aacA4, aac6'-lla, ermA, ermB, ermC, mecA, vanA, vanB, vanC, satA, aac(6'-aph(2''), aad(6'), vat, vga, msrA, sul and int are also disclosed. The above microbial species, genera and resistance genes are all clinically relevant and commonly encountered in a variety of clinical specimens. These DNA-based assays are rapid, accurate and can be used in clinical microbiology laboratories for routine diagnosis. These novel diagnostic tools should be useful to improve the speed and accuracy of diagnosis of microbial infections, thereby allowing more effective treatments. Diagnostic kits for (i) the universal detection and quantification of bacteria, and/or (ii) the detection, identification and quantification of the above-mentioned bacterial and fungal species and/or genera, and/or (iii) the detection, identification and quantification of the above-mentioned antibiotic resistance genes are also claimed.

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#### TITLE OF THE INVENTION

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SPECIES-SPECIFIC, GENUS-SPECIFIC AND UNIVERSAL DNA PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL AND FUNGAL PATHOGENS AND ASSOCIATED ANTIBIOTIC RESISTANCE GENES FROM CLINICAL SPECIMENS FOR DIAGNOSIS IN MICROBIOLOGY LABORATORIES

#### **BACKGROUND OF THE INVENTION**

## Classical methods for the identification and susceptibility testing of bacteria

Bacteria are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, at least two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan system from Dade Diagnostics Corp. and the Vitek system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these faster systems always require the primary isolation of the bacteria as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. The fastest identification system, the autoSCAN-Walk-Away™ system (Dade Diagnostics Corp.) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5.5 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than Enterobacteriaceae (Croizé J., 1995, Lett. Infectiol. 10:109-113; York et al., 1992, J. Clin. Microbiol. 30:2903-2910). For Enterobacteriaceae, the percentage of non-conclusive identifications was 2.7 to 11.4%.

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A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the most frequently associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

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## Clinical specimens tested in clinical microbiology laboratories

Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and susceptibility testing.

# Conventional pathogen identification from clinical specimens

## **Urine specimens**

The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 µL of urine is streaked on plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial count of 10<sup>7</sup> CFU/L or more in urine. However, infections with less than 10<sup>7</sup> CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. 311:560-564). Importantly, approximately 80% of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10<sup>7</sup> CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koening *et al.*, 1992, J. Clin. Microbiol. 30:342-345; Pezzlo *et al.*, 1992, J. Clin. Microbiol. 30:640-684).

#### **Blood specimens**

The blood specimens received in the microbiology laboratory are always submitted for culture. Blood culture systems may be manual, semi-automated or completely automated. The BACTEC system (from Becton Dickinson) and the

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BacTAlert system (from Organon Teknika Corporation) are the two most widely used automated blood culture systems. These systems incubate blood culture bottles under optimal conditions for bacterial growth. Bacterial growth is monitored continuously to detect early positives by using highly sensitive bacterial growth detectors. Once growth is detected, a Gram stain is performed directly from the blood culture and then used to inoculate nutrient agar plates. Subsequently, bacterial identification and susceptibility testing are carried out from isolated bacterial colonies with automated systems as described previously. The bottles are normally reported as negative if no growth is detected after an incubation of 6 to 7 days. Normally, the vast majority of blood cultures are reported negative. For example, the percentage of negative blood cultures at the microbiology laboratory of the CHUL for the period February 1994–January 1995 was 93.1% (Table 3).

## Other clinical samples

Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial, pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3).

Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial pathogens potentially associated with the infection are purified from the contaminants and then identified as described previously. Of course, the universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non sterile sites. On the other hand, DNA-based assays for species or genus detection and identification as well as for the detection of antibiotic resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

### **DNA-based assays with any clinical specimens**

There is an obvious need for rapid and accurate diagnostic tests for bacterial detection and identification directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The DNA probes and amplification primers which are objects of the present invention are applicable for bacterial or fungal detection and identification directly from any clinical specimens such as blood cultures, blood, urine, sputum, cerebrospinal fluid, pus and other type of specimens (Table 3). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since

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these tests are performed in around only one hour, they provide the clinicians with new diagnostic tools which should contribute to increase the efficiency of therapies with antimicrobial agents. Clinical specimens from organisms other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock and others) may also be tested with these assays.

## A high percentage of culture negative specimens

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Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would also be desirable, in addition to identify bacteria at the species or genus level in a given specimen, to screen out the high proportion of negative clinical specimens with a test detecting the presence of any bacterium (i.e. universal bacterial detection). Such a screening test may be based on the DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for bacteria would give a positive amplification signal with this assay.

## Towards the development of rapid DNA-based diagnostic tests

A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antibiotic resistance genes from clinical samples (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). There is no need for culture of the bacterial pathogens, hence the organisms can be detected directly from clinical samples, thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for bacterial identification than currently used phenotypic identification systems which are based on biochemical tests. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as Mycobacterium tuberculosis, Chlamydia trachomatis, Neisseria gonorrhoeae as well as for the detection of a variety of viruses (Podzorski and Persing, Molecular detection and identification of microorganisms, In: P. Murray et al., 1995, Manual of Clinical Microbiology, ASM press, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention: *Staphylococcus* spp. (US patent application serial No. US 5 437 978), *Neisseria* spp. (US patent application

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serial No. US 5 162 199 and European patent application serial No. EP 0 337 896 131) and *Listeria monocytogenes* (US patent applications serial Nos US 5 389 513 and US 5 089 386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from those described in the present invention.

Although there are diagnostic kits or methods already used in clinical microbiology laboratories, there is still a need for an advantageous alternative to the conventional culture identification methods in order to improve the accuracy and the speed of the diagnosis of commonly encountered bacterial infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the bacterial genotype (e.g. DNA level) is more stable than the bacterial phenotype (e.g. metabolic level).

Knowledge of the genomic sequences of bacterial and fungal species continuously increases as testified by the number of sequences available from databases. From the sequences readily available from databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial or fungal pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial or fungal pathogens, (iii) the universal detection of bacterial or fungal pathogens and/or (iv) the specific detection and identification of antibiotic resistance genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

In our co-pending U.S. (N.S. 08/526,840) and PCT (PCT/CA/95/00528) patent applications, we described DNA sequences suitable for (i) the species-specific detection and identification of 12 clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of 17 antibiotic resistance genes. This co-pending application described proprietary DNA sequences and DNA sequences selected from databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from these sequences. All the nucleic acid sequences described in this patent application enter the composition of diagnostic kits and methods capable of a) detecting the presence of bacteria, b) detecting specifically the presence of 12 bacterial species and 17 antibiotic resistance genes. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and antibiotic resistance genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their

resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antibiotic resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent application.

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## STATEMENT OF THE INVENTION

It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

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- from specific microbial species or genera selected from the group consisting of Streptococcus species, Streptococcus agalactiae, Staphylococcus species, Staphylococcus saprophyticus, Enterococcus species, Enterococcus faecium, Neisseria species, Neisseria meningitidis, Listeria monocytogenes, Candida species and Candida albicans

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- from an antibiotic resistance gene selected from the group consisting of  $bla_{tem}$ ,  $bla_{rob}$ ,  $bla_{shv}$ ,  $bla_{oxa}$ ,  $bla_{ox$ 

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- from any bacterial species

in any sample suspected of containing said nucleic acids,

wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probe or primers;

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said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any bacterial species, specific microbial species or genus and antibiotic resistance gene.

In a specific embodiment, a similar method directed to each specific microbial species or genus detection and identification, antibiotic resistance genes detection, and universal bacterial detection, separately, is provided.

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In a more specific embodiment, the method makes use of DNA fragments (proprietary fragments and fragments obtained from databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted bacterial or fungal nucleic acids.

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In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers.

The proprietary oligonucleotides (probes and primers) are also another object of the invention.

Diagnostic kits comprising probes or amplification primers for the detection of

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a microbial species or genus selected from the group consisting of *Streptococcus* species, *Streptococcus agalactiae*, *Staphylococcus* species, *Staphylococcus* species, *Staphylococcus* saprophyticus, *Enterococcus* species, *Enterococcus faecium*, *Neisseria* species, *Neisseria meningitidis*, *Listeria monocytogenes*, *Candida* species and *Candida* albicans are also objects of the present invention.

Diagnostic kits further comprising probes or amplification primers for the detection of an antibiotic resistance gene selected from the group consisting of  $bla_{tem}$ ,  $bla_{rob}$ ,  $bla_{shv}$ ,  $bla_{oxa}$ ,  $bla_$ 

Diagnostic kits further comprising probes or amplification primers for the detection of any bacterial or fungal species, comprising or not comprising those for the detection of the specific microbial species or genus listed above, and further comprising or not comprising probes and primers for the antibiotic resistance genes listed above, are also objects of this invention.

In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or genus, antibiotic resistance genes and for the detection of any bacterium.

In the above methods and kits, amplification reactions may include a) polymerase chain reaction (PCR), b) ligase chain reaction, c) nucleic acid sequence-based amplification, d) self-sustained sequence replication, e) strand displacement amplification, f) branched DNA signal amplification, g) transcription-mediated amplification, h) cycling probe technology (CPT) i) nested PCR, or j) multiplex PCR.

In a preferred embodiment, a PCR protocol is used as an amplification reaction.

In a particularly preferred embodiment, a PCR protocol is provided, comprising, for each amplification cycle, an annealing step of 30 seconds at 45-55°C and a denaturation step of only one second at 95°C, without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with all selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific and antibiotic resistance gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

We aim at developing a rapid test or kit to discard rapidly all the samples which are negative for bacterial cells and to subsequently detect and identify the above bacterial and/or fungal species and genera and to determine rapidly the bacterial resistance to antibiotics. Although the sequences from the selected antibiotic resistance genes are available from databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current gold standard diagnostic methods based on bacterial

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cultures. Using an amplification method for the simultaneous bacterial detection and identification and antibiotic resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure will save lives by optimizing treatment, will diminish antibiotic resistance because less antibiotics will be prescribed, will reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and decrease the time and costs associated with clinical laboratory testing.

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In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from proprietary fragments or from databases. DNA fragments selected from databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

It is clear to the individual skilled in the art that other oligonucleotide sequences appropriate for (i) the universal bacterial detection, (ii) the detection and identification of the above microbial species or genus and (iii) the detection of antibiotic resistance genes other than those listed in Annex VI may also be derived from the proprietary fragments or selected database sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones we have chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific and resistance gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annex VI which are suitable for diagnostic purposes. When a proprietary fragment or a database sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

Since a high percentage of clinical specimens are negative for bacteria (Table

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3), DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and database sequences. The amplification primers were selected from a gene highly conserved in bacteria and fungi, and are used to detect the presence of any bacterial pathogen in clinical specimens in order to determine rapidly (approximately one hour) whether it is positive or negative for bacteria. The selected gene, designated tuf, encodes a protein (EF-Tu) involved in the translational process during protein synthesis. The tuf gene sequence alignments used to derive the universal primers include both proprietary and database sequences (Example 1 and Annex I). This strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for bacteriological testing. Tables 4, 5 and 6 provide a list of the bacterial or fungal species used to test the specificity of PCR primers and DNA probes. Table 7 gives a brief description of each species-specific, genus-specific and universal amplification assays which are objects of the present invention. Tables 8, 9 and 10 provide some relevant information about the proprietary and database sequences selected for diagnostic puposes.

#### **DETAILED DESCRIPTION OF THE INVENTION**

Development of species-specific, genus-specific, universal and antibiotic resistance gene-specific DNA probes and amplification primers for microorganisms

# Selection from databases of sequences suitable for diagnostic purposes

In order to select sequences which are suitable for species-specific or genusspecific detection and identification of bacteria or fungi or, alternatively, for the universal detection of bacteria, the database sequences (GenBank, EMBL and Swiss-Prot) were chosen based on their potential for diagnostic purposes according to sequence information and computer analysis performed with these sequences. Initially, all sequence data available for the targeted microbial species or genus were carefully analyzed. The gene sequences which appeared the most promising for diagnostic purposes based on sequence information and on sequence comparisons with the corresponding gene in other microbial species or genera performed with the Genetics Computer Group (GCG, Wisconsin) programs were selected for testing by PCR. Optimal PCR amplification primers were chosen from the selected database sequences with the help of the Oligo™ 4.0 primer analysis software (National Biosciences Inc., Plymouth, Minn.). The chosen primers were tested in PCR assays for their specificity and ubiquity for the target microbial species or genus. In general, the identification of database sequences from which amplification primers suitable for species-specific or genus-specific detection and identification were selected involved the computer analysis and PCR testing of several candidate gene sequences before

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obtaining a primer pair which is specific and ubiquitous for the target microbial species or genus. Annex VI provides a list of selected specific and ubiquitous PCR primer pairs. Annexes I to V and Examples 1 to 4 illustrate the strategy used to select genus-specific, species-specific and universal PCR primers from *tuf* sequences or from the *rec*A gene.

# Oligonucleotide primers and probes design and synthesis

The DNA fragments sequenced by us or selected from databases (GenBank and EMBL) were used as sources of oligonucleotides for diagnostic purposes. For this strategy, an array of suitable oligonucleotide primers or probes derived from a variety of genomic DNA fragments (size of more than 100 bp) selected from databases were tested for their specificity and ubiquity in PCR and hybridization assays as described later. It is important to note that the database sequences were selected based on their potential for being species-specific, genus-specific or universal for the detection of bacteria or fungi according to available sequence information and extensive analysis and that, in general, several candidate database sequences had to be tested in order to obtain the desired specificity, ubiquity and sensitivity.

Oligonucleotide probes and amplification primers derived from species-specific fragments selected from database sequences were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division). Prior to synthesis, all oligonucleotides (probes for hybridization and primers for DNA amplification) were evaluated for their suitability for hybridization or DNA amplification by polymerase chain reaction (PCR) by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo<sup>TM</sup> 4.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

The oligonucleotide primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of bacteria, (ii) the species-specific detection and identification of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae* and *Candida albicans* (iii) the genus-specific detection of *Streptococcus* species, *Enterococcus* species, *Staphylococcus* species and *Neisseria* species or (iv) the detection of the 26 above-mentioned clinically important antibiotic resistance genes.

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Variants for a given target bacterial gene are naturally occurring and are attributable to sequence variation within that gene during evolution (Watson et al., 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, CA; Lewin, 1989, Genes IV, John Wiley & Sons, New York, NY). For example, different strains of the same bacterial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant bacterial or fungal DNA sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant bacterial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

## Sequencing of tuf sequences from a variety of bacterial and fungal species

The nucleotide sequence of a portion of tuf genes was determined for a variety of bacterial and fungal species. The amplification primers SEQ ID NOs: 107 and 108, which amplify a tuf gene portion of approximately 890 bp, were used for the sequencing of bacterial tuf sequences. The amplification primers SEQ ID NOs: 109 and 172, which amplify a tuf gene portion of approximately 830 bp, were used for the sequencing of fungal tuf sequences. Both primer pairs can amplify tufA and tufB genes. This is not surprising because these two genes are nearly identical. For example, the entire tufA and tufB genes from E. coli differ at only 13 nucleotide positions (Neidhardt et al., 1996, Escherichia coli and Salmonella: Cellular and Molecular Biology, 2<sup>nd</sup> ed., American Society for Microbiology Press, Washington, D.C.). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of tuf sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The amplification primers SEQ ID NOs: 107 and 108 could be used to amplify the tuf genes from any bacterial species. The amplification primers SEQ ID NOs: 109 and 172 could be used to amplify the tuf genes from any fungal species.

The *tuf* genes were amplified directly from bacterial or yeast cultures using the following amplification protocol: One  $\mu$ L of cell suspension was transferred directly to

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19  $\mu$ L of a PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 1  $\mu$ M of each of the 2 primers, 200  $\mu$ M of each of the four dNTPs, 0.5 unit of Taq DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 96°C followed by 30-35 cycles of 1 min at 95°C for the denaturation step, 1 min at 30-50°C for the annealing step and 1 min at 72°C for the extension step. Subsequently, twenty microliters of the PCRamplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The gel was then visualized by staining with methylene blue (Flores et al., 1992, Biotechniques, 13:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product (i.e. approximately 890 or 830 bp for bacterial or fungal tuf sequences, respectively) was excised from the agarose gel and purified using the QlAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, CA). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the tuf genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase® Terminator Double-stranded DNA Sequencing Kit (Perkin-Elmer Corp., Applied Biosystems Division, Foster City, CA). The sequencing reactions were all performed by using the amplification primers (SEQ ID NOs: 107 to 109 and 172) and 100 ng per reaction of the gel-purified amplicon. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artefacts, we have sequenced two preparations of the gel-purified tuf amplification product originating from two independent PCR amplifications. For all target microbial species, the sequences determined for both amplicon preparations were identical. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The tuf sequences determined using the above strategy are all in the Sequence Listing (i.e. SEQ ID NOs:118 to 146). Table 13 gives the originating microbial species and the source for each tuf sequence in the Sequence Listing.

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The alignment of the *tuf* sequences determined by us or selected from databases reveals clearly that the length of the sequenced portion of the *tuf* genes is variable. There may be insertions or deletions of several amino acids. This explains why the size of the sequenced *tuf* amplification product was variable for both bacterial and fungal species. Among the *tuf* sequences determined by our group, we found insertions and deletions adding up to 5 amino acids or 15 nucleotides. Consequently, the nucleotide positions indicated on top of each of Annexes I to V do not correspond for *tuf* sequences having insertions or deletions.

It should also be noted that the various tuf sequences determined by us

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occasionally contain degenerescences. These degenerated nucleotides correspond to sequence variations between *tufA* and *tufB* genes because the amplification primers amplify both *tuf* genes. These nucleotide variations were not attributable to nucleotide misincorporations by the *taq* DNA polymerase because the sequence of both strands were identical and also because the sequences determined with both preparations of the gel-purified *tuf* amplicons were identical.

# The selection of amplification primers from tuf sequences

The *tuf* sequences determined by us or selected from databases were used to select PCR primers for (i) the universal detection of bacteria, (ii) the genus-specific detection and identification of *Enterococcus* spp. and *Staphylococcus* spp. and (iii) the species-specific detection and identification of *Candida albicans*. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various *tuf* sequences. For more details about the selection of PCR primers from *tuf* sequences, please refer to Examples 1 to 3 and Annexes I to IV.

## The selection of amplification primers from recA

The comparison of the nucleotide sequence for the *recA* gene from various bacterial species including 5 species of streptococci allowed the selection of *Streptococcus*-specific PCR primers. For more details about the selection of PCR primers from *recA*, please refer to Example 4 and Annex V.

# 20 <u>DNA fragment isolation from Staphylococcus saprophyticus by arbitrarily primed PCR</u>

DNA sequences of unknown coding potential for the species-specific detection and identification of *Staphylococcus saprophyticus* were obtained by the method of arbitrarily primed PCR (AP-PCR).

AP-PCR is a method which can be used to generate specific DNA probes for microorganisms (Fani *et al.*, 1993, Mol. Ecol. 2:243-250). A description of the AP-PCR protocol used to isolate a species-specific genomic DNA fragment from *Staphylococcus saprophyticus* follows. Twenty different oligonucleotide primers of 10 nucleotides in length (all included in the AP-PCR kit OPAD (Operon Technologies, Inc., Alameda, CA)) were tested systematically with DNAs from 3 bacterial strains of *Staphylococcus saprophyticus* (all obtained from the American Type Culture Collection (ATCC): numbers 15305, 35552 and 43867) as well as with DNA from four other staphylococcal species (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970 and *Staphylococcus hominis* ATCC 35982). For all bacterial species, amplification was performed from a bacterial suspension adjusted to a standard 0.5 McFarland which corresponds to approximately 1.5 x  $10^8$  bacteria/mL. One  $\mu$ L of the standardized bacterial suspension was transferred directly to  $19 \mu$ L of a PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MqCl<sub>2</sub>,

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 $1.2~\mu\text{M}$  of only one of the 20 different AP-PCR primers OPAD, 200  $\mu\text{M}$  of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler (MJ Research Inc.) as follows: 3 min at 96°C followed by 35 cycles of 1 min at 95°C for the denaturation step, 1 min at 32°C for the annealing step and 1 min at 72°C for the extension step. A final extension step of 7 min at 72°C was made after the 35 cycles to ensure complete extension of PCR products. Subsequently, twenty microliters of the PCR amplified mixture were resolved by electrophoresis in a 2% agarose gel containing 0.25  $\mu$ g/mL of ethidium bromide. The size of the amplification products was estimated by comparison with a 50-bp molecular weight ladder.

Amplification patterns specific for *Staphylococcus saprophyticus* were observed with the AP-PCR primer OPAD-9 (SEQ ID NO: 25). Amplification with this primer consistently showed a band corresponding to a DNA fragment of approximately 450 bp for all *Staphylococcus saprophyticus* strains tested but not for any of the four other staphylococcal species tested. This species-specific pattern was confirmed by testing 10 more clinical isolates of *S. saprophyticus* selected from the culture collection of the microbiology laboratory of the CHUL as well as strains selected from the gram-positive bacterial species listed in Table 5.

The band corresponding to the approximately 450 bp amplicon which was specific and ubiquitous for *S. saprophyticus* based on AP-PCR was excised from the agarose gel and purified using the QlAquick<sup>™</sup> gel extraction kit (QlAGEN Inc.). The gel-purified DNA fragment was cloned into the T/A cloning site of the pCR 2.1<sup>™</sup> plasmid vector (Invitrogen Inc.) using T4 DNA ligase (New England BioLabs). Recombinant plasmids were transformed into *E. coli* DH5α competent cells using standard procedures. Plasmid DNA isolation was done by the method of Birnboim and Doly (Nucleic Acids Res. 7:1513-1523) for small-scale preparations. All plasmid DNA preparations were digested with the *Eco*RI restriction endonuclease to ensure the presence of the approximately 450 bp AP-PCR insert into the recombinant plasmids. Subsequently, a large-scale and highly purified plasmid DNA preparation was performed from two selected clones shown to carry the AP-PCR insert by using the QlAGEN plasmid purification kit. These plasmid preparations were used for automated DNA sequencing.

Both strands of the AP-PCR insert from the two selected clones were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers, by using an Applied Biosystems automated DNA sequencer as described previously. The analysis of the obtained sequences revealed that the DNA sequences for both strands from each clone were 100% complementary. Furthermore, it showed that the entire sequence determined for each clone were both identical. These sequencing data confirm the 100% accuracy for the determined 438

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bp sequence (SEQ ID NO: 29). Optimal amplification primers have been selected from the sequenced AP-PCR Staphylococcus saprophyticus DNA fragment with the help of the primer analysis software Oligo™ 4.0. The selected primer sequences have been tested in PCR assays to verify their specificity and ubiquity (Table 7). These PCR primers were specific since there was no amplification with DNA from bacterial species other than S. saprophyticus selected from Tables 4 and 5. Furthermore, this assay was ubiquitous since 245 of 260 strains of S. saprophyticus were efficiently amplified with this PCR assay. When used in combination with another S. saprophyticus-specific PCR assay, which is an object of our co-pending U.S. (N.S. 08/526,840) and PCT (PCT/CA/95/00528) patent applications, the ubiquity reaches 100% for these 260 strains.

### **DNA** amplification

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For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo™ 4.0 software to verify that they are good candidates for PCR amplification.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the bacterial genome are used to amplify exponentially in vitro the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing et al, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

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Briefly, the PCR protocols were as follow: Treated clinical specimens or standardized bacterial or fungal suspensions (see below) were amplified in a 20  $\mu$ L PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM MgCl<sub>2</sub>,  $0.4~\mu\mathrm{M}$  of each primer, 200  $\mu\mathrm{M}$  of each of the four dNTPs and 0.5 unit of Tag DNA polymerase (Promega) combined with the TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, CA). The TaqStart™ antibody, which is a neutralizing monoclonal antibody to Taq DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg et al., 1994, Biotechniques 16:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the bacterial cells and eliminate the PCR inhibitory effects (see example 11 for urine specimen preparation). For amplification from bacterial or fungal cultures, the samples were added directly to the PCR amplification mixture without any pre-treatment step (see example 10). Primer sequences derived from highly conserved regions of the bacterial 16S ribosomal RNA gene were used to provide an internal control for all PCR reactions. Alternatively, the

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internal control was derived from sequences not found in microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. The internal control derived from rRNA was also useful to monitor the efficiency of bacterial lysis protocols.

PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 second at 55°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.) and subsequently analyzed by standard ethidium bromide-stained agarose gel electrophoresis. The number of cycles performed for the PCR assays varies according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after amplification (e.g. TaqMan™ system from Perkin Elmer or Amplisensor™ from Biotronics). Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated (Example 14).

Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any species-specific or genus-specific DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus detection and identification may be derived from the amplicons produced by the universal amplification assay. The oligonucleotide probes may be labeled with biotin or with digoxigenin or with any other reporter molecules.

To assure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York). The

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concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and  $MgCl_2$  are 0.1-1.5  $\mu$ M and 1.5-3.5 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples 9 to 14.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA) and cycling probe technology (CPT) (Lee *et al.*, 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, MA; Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase rapidity and sensitivity of the tests. Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR and derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antibiotic resistance gene sequences included in this document are also under the scope of this invention.

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#### Hybridization assays with oligonucleotide probes

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In hybridization experiments, single-stranded oligonucleotides (size less than 100 nucleotides) have some advantages over DNA fragment probes for the detection of bacteria, such as ease of synthesis in large quantities, consistency in results from batch to batch and chemical stability. Briefly, for the hybridizations, oligonucleotides were 5' end-labeled with the radionucleotide γ-32P(dATP) using T4 polynucleotide kinase (Pharmacia) (Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The unincorporated radionucleotide was removed by passing the labeled oligonucleotide through a Sephadex G-50<sup>TM</sup> column. Alternatively, oligonucleotides were labeled with biotin, either enzymatically at their 3' ends or incorporated directly during synthesis at their 5' ends, or with digoxigenin. It will be appreciated by the person skilled in the art that labeling means other than the three above labels may be used.

Each oligonucleotide probe was then tested for its specificity by hybridization to DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6. All of the bacterial or fungal species tested were likely to be pathogens associated

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with common infections or potential contaminants which can be isolated from clinical specimens. Each target DNA was released from bacterial cells using standard chemical treatments to lyse the cells (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Subsequently, the DNA was denatured by conventional methods and then irreversibly fixed onto a solid support (e.g. nylon or nitrocellulose membranes) or free in solution. The fixed single-stranded target DNAs were then hybridized with the oligonucleotide probe cells (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Prehybridization conditions were in 1 M NaCl + 10% dextran sulfate + 1% SDS + 100 μg/mL salmon sperm DNA at 65°C for 15 min. Hybridization was performed in fresh pre-hybridization solution containing the labeled probe at 65°C overnight. Posthybridization washing conditions were as follows: twice in 3X SSC containing 1% SDS, twice in 2X SSC containing 1% SDS and twice in 1X SSC containing 1% SDS (all of these washes were at 65°C for 15 min), and a final wash in 0.1X SSC containing 1% SDS at 25°C for 15 min. Autoradiography of washed filters allowed the detection of selectively hybridized probes. Hybridization of the probe to a specific target DNA indicated a high degree of similarity between the nucleotide sequence of these two DNAs because of the high stringency of the washes.

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An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus from which it was isolated. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes recognized most or all isolates of the target species or genus) by hybridization to microbial DNAs from clinical isolates of the species or genus of interest including ATCC strains. The DNAs from strains of the target species or genus were denatured, fixed onto nylon membranes and hybridized as described above. Probes were considered ubiquitous when they hybridized specifically with the DNA from at least 80% of the isolates of the target species or genus.

## Specificity and ubiquity tests for oligonucleotide primers and probes

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The specificity of oligonucleotide primers and probes, derived either from the DNA fragments sequenced by us or selected from databases, was tested by amplification of DNA or by hybridization with bacterial or fungal species selected from those listed in Tables 4, 5 and 6, as described in the two previous sections. Oligonucleotides found to be specific were subsequently tested for their ubiquity by amplification (for primers) or by hybridization (for probes) with bacterial DNAs from isolates of the target species or genus. Results for specificity and ubiquity tests with the oligonucleotide primers are summarized in Table 7. The specificity and ubiquity of the PCR assays using the selected amplification primer pairs were tested directly from cultures (see Examples 9 and 10) of bacterial or fungal species.

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The various species-specific and genus-specific PCR assays which are objects of the present invention are all specific. For the PCR assays specific to bacterial species or genus, this means that DNA isolated from a wide variety of bacterial species, other than that from the target species or genus and selected from Tables 4 and 5, could not be amplified. For the PCR assay specific to *Candida albicans*, it means there was no amplification with genomic DNA from the fungal species listed in Table 6 as well as with a variety of bacterial species selected from Tables 4 and 5.

The various species-specific and genus-specific PCR assays which are objects of the present invention are also all ubiquitous (Table 7). (i) The species-specific PCR assays for E. faecium, L. monocytogenes, S. saprophyticus, S. agalactiae and C. albicans amplified genomic DNA from all or most strains of the target species tested. which were obtained from various sources and which are representative of the diversity within each target species (Table 7). The species identification of all of these strains was based on classical biochemical methods which are routinely used in clinical microbiology laboratories. (ii) The genus-specific PCR assays specific for Enterococcus spp., Staphylococcus spp., Streptococcus spp. and Neisseria spp. amplified genomic DNA from all or most strains of the target genus tested, which represent all clinically important bacterial species for each target genus. These strains were obtained from various sources and are representative of the diversity within each target genus. Again, the species identification of all of these strains was based on classical biochemical methods which are routinely used in clinical microbiology laboratories. More specifically, the four genus-specific PCR assays amplified the following species: (1) The Enterococcus-specific assay amplified efficiently DNA from all of the 11 enterococcal species tested including E. avium, E. casseliflavus, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. mundtii and E. raffinosus. (2) The Neisseria-specific assay amplified efficiently DNA from all of the 12 neisserial species tested including N. canis, N. cinerea, N. elongata, N. flavescens, N. gonorrhoeae, N. lactamica, N. meningitidis, N. mucosa, N. polysaccharea, N. sicca, N. subflava and N. weaveri. (3) The Staphylococcus-specific assay amplified efficiently DNA from 13 of the 14 staphylococcal species tested S. aureus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. schleiferi, S. simulans, S. warneri and S. xylosus. The staphylococcal species which could not be amplified is S. sciuri. (4) Finally, the Streptococcus-specific assay amplified efficiently DNA from all of the 22 streptococcal species tested including S. agalactiae, S. anginosus, S. bovis, S. constellatus, S. crista, S. dysgalactiae, S. equi, S. gordonii, S. intermedius, S. mitis, S. mutans, S. oralis, S. parasanguis, S. pneumoniae, S. pyogenes, S. salivarius, S. sanguis, S. sabrinus, S. suis, S. uberis, S. vestibularis and S. viridans. On the other hand, the Streptococcus-specific assay did not amplify 3 out of 9 strains

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of *S. mutans* and 1 out of 23 strains of *S. salivarius*, thereby showing a slight lack of ubiquity for these two streptococcal species.

All specific and ubiquitous amplification primers for each target microbial species or genus or antibiotic resistance gene investigated are listed in Annex VI. Divergence in the sequenced DNA fragments can occur, insofar as the divergence of these sequences or a part thereof does not affect the specificity of the probes or amplification primers. Variant bacterial DNA is under the scope of this invention.

The PCR amplification primers listed in Annex VI were all tested for their specificity and ubiquity using reference strains as well as clinical isolates from various geographical locations. The 351 reference strains used to test the amplification and hybridization assays (Tables 4, 5 and 6) were obtained from (i) the American Type Culture Collection (ATCC): 85%, (ii) the Laboratoire de santé publique du Québec (LSPQ): 10%, (iii) the Centers for Disease Control and Prevention (CDC): 3%, (iv) the National Culture Type Collection (NCTC): 1% and (v) several other reference laboratories throughout the world: 1%. These reference strains are representative of (i) 90 gram-negative bacterial species (169 strains; Table 4), (ii) 97 gram-positive bacterial species (154 strains; Table 5) and (iii) 12 fungal species (28 strains; Table 6).

#### Antibiotic resistance genes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of bacterial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus-specific DNA-based tests, clinicians also need timely information about the ability of the bacterial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly bacterial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antibiotic resistance genes (i.e. DNA-based tests for the detection of antibiotic resistance genes). Since the sequence from the most important and common bacterial antibiotic resistance genes are available from databases, our strategy was to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of rapid DNA-based tests. The sequence from each of the bacterial antibiotic resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in the Sequence Listing. Tables 9 and 10 summarize some characteristics of the selected antibiotic resistance genes. Our approach is unique because the antibiotic resistance genes detection and the bacterial detection and identification are performed simultaneously in multiplex assays under

uniform PCR amplification conditions (Example 13).

Annex VI provides a list of all amplification primers selected from 26 clinically important antibiotic resistance genes which were tested in PCR assays. The various PCR assays for antibiotic resistance genes detection and identification were validated by testing several resistant bacterial isolates known to carry the targeted gene and obtained from various countries. The testing of a large number of strains which do not carry the targeted resistance gene was also performed to ensure that all assays were specific. So far, all PCR assays for antibiotic resistance genes are highly specific and have detected all control resistant bacterial strains known to carry the targeted gene. The results of some clinical studies to validate the array of PCR assays for the detection and identification of antibiotic resistance genes and correlate these DNA-based assays with standard antimicrobials susceptibility testing methods are presented in Tables 11 and 12.

## Universal bacterial detection

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In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture (Table 4). Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screen out the numerous negative specimens is thus useful as it saves costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the *tuf* genes (Table 8). The universal primer selection was based on a multiple sequence alignment constructed with sequences determined by us or selected from available database sequences as described in Example 1 and Annex I.

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For the identification of database sequences suitable for the universal detection of bacteria, we took advantage of the fact that the complete genome sequences for two distant microorganisms (i.e. *Mycoplasma genitalium* and *Haemophilus influenzae*) are available. A comparison of the amino acid sequence for all proteins encoded by the genome of these two distant microorganisms led to the identification of highly homologous proteins. An analysis of these homologous proteins allowed to select some promising candidates for the development of universal DNA-based assays for the detection of bacteria. Since the complete nucleotide sequence of several other microbial genomes are presently available in databases, a person skilled in the art could arrive to the same conclusions by comparing genomes sequences other than those of *Mycoplasma genitalium* and *Haemophilus influenzae*. The selected *tuf* gene encodes a protein (EF-Tu) involved in the translation process during protein synthesis. Subsequently, an extensive nucleotide sequence analysis was performed with the *tuf* gene sequences available in databases as well as with novel *tuf* sequences which we have determined as described previously. All computer analysis of amino acid and

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nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for the universal amplification of bacteria were selected with the help of the Oligo™ program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species (Annex I). Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

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The amplification conditions with the universal primers were identical to those used for the species- and genus-specific amplification assays except that the annealing temperature was 50°C instead of 55°C. This universal PCR assay was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species listed in Table 6 as well as genomic DNA from Leishmania donovani, Saccharomyces cerevisiae and human lymphocytes. None of the above eukaryotic DNA preparations 20 could be amplified by the universal assay, thereby suggesting that this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Tables 4 and 5. We found that 104 of these 116 strains could be amplified. The bacterial species which could not be amplified belong to the following genera: Corynebacterium (11 species) and Stenotrophomonas (1 species). Sequencing of the tuf genes from these bacterial species has been recently performed. This sequencing data has been used to select new universal primers which may be more ubiquitous. These primers are in the process of being tested. We also observed that for several species the annealing temperature had to be reduced to 45°C in order to get an efficient amplification. These bacterial species include Gemella morbilbrum, Listeria spp. (3 species) and Gardnerella vaginalis. It is important to note that the 95 bacterial species selected from Tables 4 and 5 to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

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## **EXAMPLES AND ANNEXES**

The following examples and annexes are intended to be illustrative of the various methods and compounds of the invention, rather than limiting the scope thereof.

The various annexes show the strategies used for the selection of amplification primers from tuf sequences or from the recA gene: (i) Annex I illustrates the strategy used for the selection of the universal amplification primers from tuf sequences. (ii) Annex II shows the strategy used for the selection of the amplification primers specific for the genus Enterococcus from tuf sequences. (iii) Annex III illustrates the strategy used for the selection of the amplification primers specific for the genus Staphylococcus from tuf sequences. (iv) Annex IV shows the strategy used for the selection of the amplification primers specific for the species Candida albicans from tuf sequences. (v) Annex V illustrates the strategy used for the selection of the amplification primers specific for the genus Streptococcus from recA sequences. (vi) Annex VI gives a list of all selected primer pairs. As shown in these annexes, the selected amplification primers may contain inosines and/or degenerescences. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York).

#### **EXAMPLES**

#### 25 **EXAMPLE 1**:

Selection of universal PCR primers from tuf sequences. As shown in Annex I, the comparison of tuf sequences from a variety of bacterial and eukaryotic species allowed the selection of PCR primers which are universal for the detection of bacteria. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. This multiple sequence alignment includes tuf sequences from 38 bacterial species and 3 eukaryotic species either determined by us or selected from databases (Table 13). A careful analysis of this multiple sequence alignment allowed the selection of primer sequences which are conserved within eubacteria but which discriminate sequences from eukaryotes, thereby permitting the universal detection of bacteria. As shown in Annex I, the selected primers contain several inosines and degenerescences. This was necessary because there is a relatively high polymorphism among bacterial tuf sequences despite the fact that this gene is highly conserved. In fact, among the tuf sequences that we determined, we found many nucleotide variations as well as some deletions and/or

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insertions of amino acids. The selected universal primers were specific and ubiquitous for bacteria (Table 7). Of the 95 most clinically important bacterial species tested, 12 were not amplified. These species belong to the genera *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). The universal primers did not amplify DNA of non-bacterial origin, including human and other types of eukaryotic DNA.

#### **EXAMPLE 2:**

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Selection of genus-specific PCR primers from tuf sequences. As shown in Annexes 2 and 3, the comparison of tuf sequences from a variety of bacterial species allowed the selection of PCR primers specific for Enterococcus spp. or for Staphylococcus spp. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. These multiple sequence alignments include the tuf sequences of four representative bacterial species selected from each target genus as well as tuf sequences from species of other closely related bacterial genera. A careful analysis of those alignments allowed the selection of oligonucleotide sequences which are conserved within the target genus but which discriminate sequences from other closely related genera, thereby permitting the genus-specific and ubiquitous detection and identification of the target bacterial genus.

For the selection of primers specific for *Enterococcus* spp. (Annex II), we have sequenced a portion of approximately 890 bp of the *tuf* genes for *Enterococcus avium*, *E. faecalis*, *E. faecium* and *E. gallinarum*. All other *tuf* sequences used in the alignment were either sequenced by us or selected from databases. The analysis of this sequence alignment led to the selection of a primer pair specific and ubiquitous for *Enterococcus* spp. (Table 7). All of the 11 enterococcal species tested were efficiently amplified and there was no amplification with genomic DNA from bacterial species of other genera.

For the selection of primers specific for *Staphylococcus* spp. (Annex III), we have also sequenced a portion of approximately 890 bp of the *tuf* genes for *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus* and *S. simulans*. All other *tuf* sequences used in the alignment were either sequenced by us or selected from databases. The analysis of this sequence alignment led to the selection of two primer pairs specific and ubiquitous for *Staphylococcus* spp. (Table 7). Annex III shows the strategy used to select one of these two PCR primer pairs. The same strategy was used to select the other primer pair. Of the 14 staphylococcal species tested, one (*S. sciuri*) could not be amplified by the *Staphylococcus*-specific PCR assays using either one of these two primer pairs, there was no amplification with DNA from species of other bacterial genera.

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#### **EXAMPLE 3:**

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Selection from tuf sequences of PCR primers specific for Candida albicans. As shown in Annex IV, the comparison of tuf sequences from a variety of bacterial and eukaryotic species allowed the selection of PCR primers specific for Candida albicans. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. This multiple sequence alignment includes tuf sequences of five representative fungal species selected from the genus Candida which were determined by our group (i.e. C. albicans, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis) as well as tuf sequences from other closely related fungal species. tuf sequences from various bacterial species were also included. A careful analysis of this sequence alignment allowed the selection of primers from the C. albicans tuf sequence; these primers discriminate sequences from other closely related Candida species and other fungal species, thereby permitting the species-specific and ubiquitous detection and identification of C. albicans (Table 7). All of 88 Candida albicans strains tested were efficiently amplified and there was no amplification with genomic DNA from other fungal or bacterial species.

## **EXAMPLE 4:**

Selection of PCR primers specific for *Streptococcus* from *recA*. As shown in Annex V, the comparison of the various bacterial *recA* gene sequences available from databases (GenBank and EMBL) was used as a basis for the selection of PCR primers which are specific and ubiquitous for the bacterial genus *Streptococcus*. Since sequences of the *recA* gene are available for many bacterial species including five species of streptococci, it was possible to choose sequences well conserved within the genus *Streptococcus* but distinct from the *recA* sequences for other bacterial genera. When there were mismatches between the *recA* gene sequences from the five *Streptococcus* species, an inosine residue was incorporated into the primer (Annex V). The selected primers, each containing one inosine and no degenerescence, were specific and ubiquitous for *Streptococcus* species (Table 7). This PCR assay amplified all of the 22 streptococcal species tested. However, the *Streptococcus*-specific assay did not amplify DNA from 3 out of 9 strains of *S. mutans* and 1 out of 3 strains of *S. salivarius*. There was no amplification with genomic DNA from other bacterial genera (Table 7).

#### **EXAMPLE 5**:

Nucleotide sequencing of DNA fragments. The nucleotide sequence of a portion of the *tuf* genes from a variety of bacterial or fungal species was determined by using the dideoxynucleotide chain termination sequencing method (Sanger *et al.*, 1977, Proc. Natl. Acad. Sci. USA. 74:5463-5467). The sequencing was performed by using an Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase® Terminator Double-stranded DNA Sequencing Kit (Perkin-Elmer Corp.,

Applied Biosystems Division, Foster City, CA). The sequencing strategy does not discriminate *tufA* and *tufB* genes because the sequencing primers hybridize efficiently to both bacterial *tuf* genes. These DNA sequences are shown in the sequence listing (SEQ ID Nos: 118 to 146). The presence of several degenerated nucleotides in the various *tuf* sequences determined by our group (Table 13) corresponds to sequence variations between *tufA* and *tufB*.

Oligonucleotide primers and probes selection. Oligonucleotide probes and amplification primers were selected from the given proprietary DNA fragments or database sequences using the Oligo™ program and were synthesized with an automated ABI DNA synthesizer (Model 391, Perkin-Elmer Corp., Applied Biosystems Division) using phosphoramidite chemistry.

#### **EXAMPLE 6:**

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Labeling of oligonucleotides for hybridization assays. Each oligonucleotide was 5' end-labeled with  $\gamma$ -32P (dATP) by the T4 polynucleotide kinase (Pharmacia) as described earlier. The label could also be non-radioactive.

Specificity test for oligonucleotide probes. All labeled oligonucleotide probes were tested for their specificity by hybridization to DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6 as described earlier. Species-specific or genus-specific probes were those hybridizing only to DNA from the microbial species or genus from which it was isolated. Oligonucleotide probes found to be specific were submitted to ubiquity tests as follows.

<u>Ubiquity test for oligonucleotide probes</u>. Specific oligonucleotide probes were then used in ubiquity tests with strains of the target species or genus including reference strains and other strains obtained from various countries and which are representative of the diversity within each target species or genus. Chromosomal DNAs from the isolates were transferred onto nylon membranes and hybridized with labeled oligonucleotide probes as described for specificity tests. The batteries of isolates constructed for each target species or genus contain reference ATCC strains as well as a variety of clinical isolates obtained from various sources. Ubiquitous probes were those hybridizing to at least 80% of DNAs from the battery of clinical isolates of the target species or genus.

#### **EXAMPLE 7:**

Same as example 6 except that a pool of specific oligonucleotide probes is used for microbial identification (i) to increase sensitivity and assure 100% ubiquity or (ii) to identify simultaneously more than one microbial species and/or genus. Microbial identification could be performed from microbial cultures or directly from any clinical specimen.

#### **EXAMPLE 8:**

Same as example 6 except that bacteria or fungi were detected directly from clinical samples. Any biological sample was loaded directly onto a dot blot apparatus and cells were lysed *in situ* for bacterial or fungal detection and identification. Blood samples should be heparizined in order to avoid coagulation interfering with their convenient loading on a dot blot apparatus.

#### **EXAMPLE 9:**

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PCR amplification. The technique of PCR was used to increase the sensitivity and the rapidity of the assays. The sets of primers were tested in PCR assays performed directly from bacterial colonies or from a standardized bacterial suspension (see Example 10) to determine their specificity and ubiquity (Table 7). Examples of specific and ubiquitous PCR primer pairs are listed in Annex VI.

Specificity and ubiquity tests for amplification primers. The specificity of all selected PCR primer pairs was tested against DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6 as described earlier. Primer pairs found specific for each species or genus were then tested for their ubiquity to ensure that each set of primers could amplify at least 90% of DNAs from a battery of isolates of the target species or genus. The batteries of isolates constructed for each species contain reference ATCC strains and various clinical isolates from around the world which are representative of the diversity within each species or genus.

Standard precautions to avoid false positive PCR results should be taken (Kwok and Higuchi, 1989, Nature, 239:237-238). Methods to inactivate PCR amplification products such as the inactivation by uracil-N-glycosylase may be used to control PCR carryover.

# 5 EXAMPLE 10:

Amplification directly from bacterial or yeast cultures. PCR assays were performed either directly from a bacterial colony or from a bacterial suspension, the latter being adjusted to a standard McFarland 0.5 (corresponds to approximately 1.5 x  $10^8$  bacteria/mL). In the case of direct amplification from a colony, a portion of a colony was transferred using a plastic rod directly into a  $20~\mu$ L PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer,  $200~\mu$ M of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart<sup>TM</sup> antibody (Clontech Laboratories Inc.). For the bacterial suspension, 1  $\mu$ L of the cell suspension was added to 19  $\mu$ L of the same PCR reaction mixture. For the identification from yeast cultures, 1  $\mu$ L of a standard McFarland 1.0 (corresponds to approximately 3.0 x  $10^8$  bacteria/mL) concentrated 100 times by centrifugation was added directly to the PCR reaction. This concentration step for yeast cells was performed because a McFarland 0.5 for yeast cells has approximately 200 times fewer cells than a McFarland 0.5 for bacterial cells.

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PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 55°C for the annealing-extension step) using a PTC-200 thermal cycler. PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 0.25  $\mu$ g/mL of ethidium bromide under UV at 254 nm. The entire PCR assay can be completed in approximately one hour.

Primer sequences derived from highly conserved regions of the bacterial 16S ribosomal RNA gene were used to provide an internal control for all PCR reactions. Alternatively, the internal control was derived from sequences not found in microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. The internal control derived from rRNA was also useful to monitor the efficiency of the bacterial lysis protocols. The internal control and the species-specific or genus-specific amplifications were performed simultaneously in multiplex PCR assays.

#### **EXAMPLE 11:**

Amplification directly from urine specimens. For PCR amplification performed directly from urine specimens, 1  $\mu$ L of urine was mixed with 4  $\mu$ L of a lysis solution containing 500 mM KCI, 100 mM tris-HCI (pH(9.0), 1% triton X-100. After incubation for at least 15 minutes at room temperature, 1  $\mu$ L of the treated urine specimen was added directly to 19  $\mu$ L of the PCR reaction mixture. The final concentration of the PCR reagents was 50 mM KCI, 10 mM Tris (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 200  $\mu$ M of each of the four dNTPs. In addition, each 20  $\mu$ L reaction contained 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart<sup>TM</sup> antibody (Clontech Laboratories Inc.).

Strategies for the internal control, PCR amplification and agarose gel detection of the amplicons are as previously described in example 10.

#### **EXAMPLE 12:**

Detection of antibiotic resistance genes. The presence of specific antibiotic resistance genes which are frequently encountered and clinically relevant is identified using the PCR amplification or hybridization protocols described previously. Specific oligonucleotides used as a basis for the DNA-based tests are selected from the antibiotic resistance gene sequences. These tests, which allow the rapid evaluation of bacterial resistance to antimicrobial agents, can be performed either directly from clinical specimens, from a standardized bacterial suspension or from a bacterial colony and should complement diagnostic tests for the universal detection of bacteria as well as for the species-specific and genus-specific microbial detection and identification.

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#### **EXAMPLE 13:**

Same as examples 10 and 11 except that assays were performed by multiplex PCR (i.e. using several pairs of primers in a single PCR reaction) to reach an ubiquity of 100% for the specific targeted pathogen(s). For more heterogeneous microbial species or genus, a combination of PCR primer pairs may be required to detect and identify all representatives of the target species or genus.

Multiplex PCR assays could also be used to (i) detect simultaneously several microbial species and/or genera or, alternatively, (ii) to simultaneously detect and identify bacterial and/or fungal pathogens and detect specific antibiotic resistance genes either directly from a clinical specimen or from bacterial cultures.

For these applications, amplicon detection methods should be adapted to differentiate the various amplicons produced. Standard agarose gel electrophoresis could be used because it discriminates the amplicons based on their sizes. Another useful strategy for this purpose would be detection using a variety of fluorescent dyes emitting at different wavelengths. The fluorescent dyes can be each coupled with a specific oligonucleotide linked to a fluorescence quencher which is degraded during amplification to release the fluorescent dyes (e.g. TaqMan™, Perkin Elmer).

## **EXAMPLE 14:**

Detection of amplification products. The person skilled in the art will appreciate 20 that alternatives other than standard agarose gel electrophoresis (Example 10) may be used for the revelation of amplification products. Such methods may be based on fluorescence polarization or on the detection of fluorescence after amplification (e.g. Amplisensor™, Biotronics; TaqMan™, Perkin-Elmer Corp.) or other labels such as biotin (SHARP Signal™ system, Digene Diagnostics). These methods are quantitative and may be automated. One of the amplification primers or an internal oligonucleotide probe specific to the amplicon(s) derived from the species-specific, genus-specific or universal DNA fragments is coupled with the fluorescent dyes or with any other label. Methods based on the detection of fluorescence are particularly suitable for diagnostic tests since they are rapid and flexible as fluorescent dyes emitting at different wavelengths are available.

## **EXAMPLE 15:**

Species-specific, genus-specific, universal and antibiotic resistance gene amplification primers can be used in other rapid amplification procedures such as the ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), cycling probe technology (CPT) and branched DNA (bDNA) or any other methods to increase the sensitivity of the test. Amplifications can be performed from isolated bacterial cultures or directly from any clinical specimen. The scope of this invention is therefore not limited to the use of the

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DNA sequences from the enclosed Sequence Listing for PCR only but rather includes the use of any procedures to specifically detect bacterial DNA and which may be used to increase rapidity and sensitivity of the tests.

#### **EXAMPLE 16:**

A test kit would contain sets of probes specific for each microbial species or genus as well as a set of universal probes. The kit is provided in the form of test components, consisting of the set of universal probes labeled with non-radioactive labels as well as labeled species- or genus-specific probes for the detection of each pathogen of interest in specific types of clinical samples. The kit will also include test reagents necessary to perform the pre-hybridization, hybridization, washing steps and hybrid detection. Finally, test components for the detection of known antibiotic resistance genes (or derivatives therefrom) will be included. Of course, the kit will include standard samples to be used as negative and positive controls for each hybridization test.

Components to be included in the kits will be adapted to each specimen type and to detect pathogens commonly encountered in that type of specimen. Reagents for the universal detection of bacteria will also be included. Based on the sites of infection, the following kits for the specific detection of pathogens may be developed:

- A kit for the universal detection of bacterial or fungal pathogens from all clinical specimens which contains sets of probes specific for highly conserved regions of the microbial genomes.
- A kit for the detection of microbial pathogens retrieved from urine samples, which contains 5 specific test components (sets of probes for the detection of *Enterococcus faecium*, *Enterococcus* species, *Staphylococcus* saprophyticus, *Staphylococcus* species and *Candida albicans*).
- A kit for the detection of respiratory pathogens which contains 3 specific test components (sets of probes for the detection of *Staphylococcus* species, *Enterococcus* species and *Candida albicans*).
- A kit for the detection of pathogens retrieved from blood samples, which contains 10 specific test components (sets of probes for the detection of Streptococcus species, Streptococcus agalactiae, Staphylococcus species, Staphylococcus saprophyticus, Enterococcus species, Enterococcus faecium, Neisseria species, Neisseria meningitidis, Listeria monocytogenes and Candida albicans). This kit can also be applied for direct detection and identification from blood cultures.
- A kit for the detection of pathogens causing meningitis, which contains 5 specific test components (sets of probes for the detection of *Streptococcus* species, *Listeria monocytogenes, Neisseria meningitidis, Neisseria* species and *Staphylococcus* species).

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- A kit for the detection of clinically important antibiotic resistance genes which contains sets of probes for the specific detection of at least one of the 26 following genes associated with antibiotic resistance:  $bla_{tem}$ ,  $bla_{rob}$ ,  $bla_{shv}$ ,  $bla_{oxa}$ 

- Other kits adapted for the detection of pathogens from skin, abdominal wound or any other clinically relevant infections may also be developed.

#### **EXAMPLE 17:**

Same as example 16 except that the test kits contain all reagents and controls to perform DNA amplification assays. Diagnostic kits will be adapted for amplification by PCR (or other amplification methods) performed directly either from clinical specimens or from microbial cultures. Components required for (i) universal bacterial detection, (ii) species-specific and genus-specific bacterial and/or fungal detection and identification and (iii) detection of antibiotic resistance genes will be included.

Amplification assays could be performed either in tubes or in microtitration plates having multiple wells. For assays in plates, the wells will contain the specific amplification primers and control DNAs and the detection of amplification products will be automated. Reagents and amplification primers for universal bacterial detection will be included in kits for tests performed directly from clinical specimens. Components required for species-specific and genus-specific bacterial and/or fungal detection and identification as well as for the simultaneous antibiotic resistance genes detection will be included in kits for testing directly from bacterial or fungal cultures as well as in kits

The kits will be adapted for use with each type of specimen as described in example 16 for hybridization-based diagnostic kits.

for testing directly from any type of clinical specimen.

#### **EXAMPLE 18:**

It is understood that the use of the probes and amplification primers described in this invention for bacterial and/or fungal detection and identification is not limited to clinical microbiology applications. In fact, we feel that other sectors could also benefit from these new technologies. For example, these tests could be used by industries for quality control of food, water, air, pharmaceutical products or other products requiring microbiological control. These tests could also be applied to detect and identify bacteria or fungi in biological samples from organisms other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock and others). These diagnostic tools could also be very useful for research purposes including clinical trials and epidemiological studies.

This invention has been described herein above, and it is readily apparent that modifications can be made thereto without departing from the spirit of this invention. These modifications are under the scope of this invention, as defined in the appended claims.

Table 1. Distribution (%) of nosocomial pathogens for various human infections in USA (1990-1992)¹.

| Pathogen                   | UTI <sup>2</sup> | SSI3 | BSI⁴ | Pneumonia      | CSF |
|----------------------------|------------------|------|------|----------------|-----|
| Escherichia coli           | 27               | 9    | 5    | 4              | 2   |
| Staphylococcus aureus      | 2                | 21   | 17   | 21             | 2   |
| Staphylococcus epidermidis | 2                | 6    | 20   | 0              | 1   |
| Enterococcus faecalis      | 16               | 12   | 9    | 2              | 0   |
| Enterococcus faecium       | 1                | 1    | 0    | 0              | 0   |
| Pseudomonas aeruginosa     | 12               | 9    | 3    | 18             | 0   |
| Klebsiella pneumoniae      | 7                | 3    | 4    | 9              | 0   |
| Proteus mirabilis          | 5                | 3    | 1    | 2              | 0   |
| Streptococcus pneumoniae   | 0                | 0    | 3    | 1              | 18  |
| Group B Streptococci       | 1                | 1    | 2    | 1              | 6   |
| Other Streptococci         | 3                | 5    | 2    | 1              | 3   |
| Haemophilus influenzae     | 0 ~~             | 0    | 0    | * · · · 6      | 45  |
| Neisseria meningitidis     | <b>o</b> ***     | 0    | 0    | <sup>3</sup> O | 14  |
| Listeria monocytogenes     | 0                | 0    | 0    | 0              | 3   |
| Other Enterococci          | 1                | 1    | 0    | · 0            | 0   |
| Other Staphylococci        | 2                | ž    | 8    | 13             | 20  |
| Candida albicans           | 9                | 3    | 5    | 5              | 0   |
| Other Candida              | 2                |      | 1    | 3              | 10  |
| Enterobacter spp.          | 5                | 7    | 4    | 12             | 2   |
| Acinetobacter spp.         | 1                | 1    | 2    | 4              | 2   |
| Citrobacter spp.           | 2                | 1    | 1    | 1              | 0   |
| Serratia marcescens        | 1                | 1    | 1    | 3              | 1   |
| Other Klebsiella           | 1                | 1    | 1    | 2              | 1   |
| Others                     | . 0              | 6    | 4    | 5              | 0   |

Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6:428-442).

<sup>&</sup>lt;sup>2</sup> Urinary tract infection.

<sup>&</sup>lt;sup>3</sup> Surgical site infection.

<sup>&</sup>lt;sup>4</sup> Bloodstream infection.

<sup>35 &</sup>lt;sup>5</sup> Cerebrospinal fluid.

Table 2. Distribution (%) of bloodstream infection pathogens in Quebec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

| 5        | Organism                                   | Quebec <sup>1</sup> | Canada <sup>2</sup> | UK         | USA⁴         | _               |              |
|----------|--|---------------------|---------------------|------------|--------------|-----------------|--------------|
|          |  |                     |                     | Community- | Hospital-    | Hospital-       |              |
|          |  | <del></del>         | <u></u>             | acquired   | acquired     | acquired        |              |
|          | E. coli                                    | 15.6                | 53.8                | 24.8       | 20.3         | 5.0             | _            |
|          | S. epidermidis and other CoNS <sup>5</sup> | 25.8                | NIe                 | 0.5        | 7.2          | 31.0            |              |
| 10       | S. aureus                                  | 9.6                 | NI                  | 9.7        | 19.4         | 16.0            |              |
|          | S. pneumoniae                              | 6.3                 | NI                  | 22.5       | 2.2          | NR <sup>7</sup> |              |
|          | E. faecalis                                | 3.0                 | NI                  | 1.0        | 4.2          | NR              |              |
|          | E. faecium                                 | 2.6                 | NI                  | 0.2        | 0.5          | NR              |              |
|          | Enterococcus                               | NR                  | NI                  | NR         | NR           | 9.0             |              |
| 15       | spp.                                       | :•                  |                     |            |              |                 |              |
| ,        | H. influenzae                              | 1.5                 | · NR                | · 3.4 ·    | 0.4          | NR              |              |
| <i>:</i> | P. aeruginosa                              | 1.5                 | 8.2                 | 1.0        | 8.2          | 3.0             | <del>:</del> |
| 4        | K. pneumoniae                              | 3.0                 | 11.2                | 3.0        | 9.2          | 4.0             | ***          |
|          | P. mirabilis                               | NR                  | 3.9                 | 2.8        | <b>5.3</b> ` | 1.0             |              |
| 20       | S. pyogenes                                | NR                  | NI                  | 1.9        | 0.9          | NR              |              |
|          | Enterobacter spp.                          | 4.1                 | 5.5                 | 0.5        | 2.3          | 4.0             |              |
|          | Candida spp.                               | 8.5                 | NI                  | NR         | 1.0          | 8.0             |              |
|          | Others                                     | 18.5                | 17.4 <sup>8</sup>   | 28.7       | 18.9         | 19.0            |              |

- Data obtained for 270 isolates collected at the Centre Hospitalier de l'Université Laval (CHUL) during a 5 month period (May to October 1995).
  - Data from 10 hospitals throughout Canada representing 941 gram-negative bacterial isolates. (Chamberland *et al.*, 1992, Clin. Infect. Dis., **15**:615-628).
  - Data from a 20-year study (1969-1988) for nearly 4000 isolates (Eykyn *et al.*, 1990, J. Antimicrob. Chemother., Suppl. C, **25**:41-58).
  - Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6:428-442).
  - <sup>5</sup> Coagulase-negative staphylococci.

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- <sup>6</sup> NI, not included. This survey included only gram-negative species.
- 35 <sup>7</sup> NR, incidence not reported for these species or genera.
  - <sup>8</sup> In this case, 17.4 stands for other gram-negative bacterial species.

Table 3. Distribution of positive and negative clinical specimens tested at the microbiology laboratory of the CHUL (February 1994 – January 1995).

|    | Clinical specimens      | No. of samples | % of positive | % of negative |
|----|-------------------------|----------------|---------------|---------------|
| 5  | and/or sites            | tested (%)     | specimens     | specimens     |
|    | Urine                   | 17,981 (54.5)  | 19.4          | 80.6          |
|    | Blood culture/marrow    | 10,010 (30.4)  | 6.9           | 93.1          |
|    | Sputum                  | 1,266 (3.8)    | 68.4          | 31.6          |
|    | Superficial pus         | 1,136 (3.5)    | 72.3          | 27.7          |
| 10 | Cerebrospinal fluid     | 553 (1.7)      | 1.0           | 99.0          |
|    | Synovial fluid          | 523 (1.6)      | 2.7           | 97.3          |
|    | Respiratory tract       | 502 (1.5)      | 56.6          | 43.4          |
|    | Deep pus                | 473 (1.4)      | 56.8          | 43.2          |
|    | Ears                    | 289 (0.9)      | 47.1          | 52.9          |
| 15 | Pleural and pericardial | 132 (0.4)      | 1.0           | 99.0          |
|    | fluid                   |                | ,             | *             |
|    | Peritoneal fluid        | 101(0.3)       | 28.6          | 71.4          |
| ·  | Total:                  | 32,966 (100.0) | 20.0          | 80.0          |

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Table 4. Gram-negative bacterial species (90) used to test the specificity of PCR primers and DNA probes (continues on next page).

|    | Bacterial species          | Number of | Bacterial species         | Number of           |
|----|----------------------------|-----------|---------------------------|---------------------|
|    |                            | reference |                           | reference           |
|    |                            | strains   |                           | strains             |
|    |                            | tested    |                           | tested <sup>a</sup> |
| 5  | Acinetobacter baumannii    | 1         | Moraxella phenylpyruvica  | 1                   |
|    | Acinetobacter Iwoffii      | 3         | Morganella morganii       | 1                   |
|    | Actinobacillus lignieresii | 1         | Neisseria animalis        | 1                   |
|    | Alcaligenes faecalis       | 1         | Neisseria canis           | 1                   |
|    | Alcaligenes odorans        | 1         | Neisseria caviae          | 1                   |
| 10 | Alcaligenes xylosoxydans   |           | Neisseria cinerea         | 1                   |
|    | subsp. denitrificans       | 1         | Neisseria cuniculi        | 1                   |
|    | Bacteroides distasonis     | 1         | Neisseria elongata        | 1                   |
|    |                            |           | subsp. <i>elongata</i>    |                     |
|    | Bacteroides fragilis       | 1         | Neisseria elongata        | 1                   |
|    | ÷ .                        |           | subsp. glycoytica         | 3                   |
|    | Bacteroides ovatus         | 1         | Neisseria flavescens      | 1                   |
| 15 | Bacteroides                | 1         | Neisseria flavescens      | 1                   |
|    | thetaiotaomicron           |           | Branham                   |                     |
|    | Bacteroides vulgatus       | 1         | Neisseria gonorrhoeae     | 18                  |
|    | Bordetella bronchiseptica  | 1         | Neisseria lactamica       | 1                   |
|    | Bordetella parapertussis   | 1         | Neisseria meningitidis    | 4                   |
| 20 | Bordetella pertussis       | 2         | Neisseria mucosa          | 2                   |
|    | Burkholderia cepacia       | 1         | Neisseria polysaccharea   | 1                   |
|    | Citrobacter amalonaticus   | 1         | Neisseria sicca           | 3                   |
|    | Citrobacter diversus       | 2         | Neisseria subflava        | 3                   |
|    | subsp. <i>koseri</i>       |           |                           |                     |
| 25 | Citrobacter freundii       | 1         | Neisseria weaveri         | 1                   |
|    | Comamonas acidovorans      | 1         | Ochrobactrum antropi      | 1                   |
|    | Enterobacter aerogenes     | 1         | Pasteurella aerogenes     | 1                   |
|    | Enterobacter               | 1         | Pasteurella multocida     | 1                   |
|    | agglomerans                |           |                           |                     |
| 30 | Enterobacter cloacae       | 1         | Prevotella melaninogenica | 1                   |
|    | Escherichia coli           | 9         | Proteus mirabilis         | 3                   |
|    | Escherichia fergusonii     | 1         | Proteus vulgaris          | 1                   |

| Bacterial species                         | Number of reference strains tested | Bacterial species               | Number of reference strains tested |
|---|------------------------------------|---------------------------------|------------------------------------|
| Escherichia hermannii                     | 1                                  | Providencia alcalifaciens       | 1                                  |
| Escherichia vulneris                      | 1                                  | Providencia rettgeri            | 1                                  |
| Flavobacterium<br>meningosepticum         | 1                                  | Providencia rustigianii         | 1                                  |
| Flavobacterium indologenes                | 1                                  | Providencia stuartii            | 1                                  |
| Flavobacterium odoratum                   | 1                                  | Pseudomonas aeruginosa          | 14                                 |
| Fusobacterium necrophorum                 | 2                                  | Pseudomonas fluorescens         | 2                                  |
| Gardnerella vaginalis                     | 1                                  | Pseudomonas stutzeri            | 1                                  |
| Haemophilus<br>haemolyticus               | 1                                  | Salmonella arizonae             | 1                                  |
| Haemophilus influenzae                    | 12                                 | Salmonella choleraesuis         | 1                                  |
| Haeṃophilus                               | . 1                                | Salmonella gallinarum           | 1 0                                |
| parahaemolyticus                          |                                    |                                 |                                    |
| Haemophilus<br>parainfluenzae             | 2                                  | Salmonella typhimurium          | 3                                  |
| Hafnia alvei                              | 1                                  | Serratia liquefaciens           | 1                                  |
| Kingella indologenes<br>subsp. suttonella | 1                                  | Serratia marcescens             | 1                                  |
| Kingella kingae                           | 1                                  | Shewanella putida               | 1                                  |
| Klebsiella ornithinolytica                | 1                                  | Shigella boydii                 | 1                                  |
| Klebsiella oxytoca                        | 1                                  | Shigella dysenteriae            | 1                                  |
| Klebsiella pneumoniae                     | 8                                  | Shigella flexneri               | 1                                  |
| Moraxella atlantae                        | 1                                  | Shigella sonnei                 | 1 ·                                |
| Moraxella catarrhalis                     | 5                                  | Stenotrophomonas<br>maltophilia | 1                                  |
| Moraxella lacunata                        | 1                                  | Yersinia enterocolitica         | 1                                  |
| Moraxella osloensis                       | 1                                  |                                 |                                    |

Most reference strains were obtained from the American Type Culture Collection (ATCC). The other reference strains were obtained from (i) the Laboratoire de Santé Publique du Québec (LSPQ), (ii) the Center for Disease Control and Prevention (CDC) and (iii) the National Culture Type Collection (NCTC).

Table 5. Gram-positive bacterial species (97) used to test the specificity of PCR primers and DNA probes (continues on next page).

|    | Bacterial species         | Number of reference strains tested | Bacterial species                   | Number of reference strains tested |
|----|---------------------------|------------------------------------|-------------------------------------|------------------------------------|
| 5  | Abiotrophia adiacens      | 1                                  | Micrococcus kristinae               | 1                                  |
|    | Abiotrophia defectiva     | 1                                  | Micrococcus luteus                  | 1                                  |
|    | Actinomyces israelii      | 1                                  | Micrococcus Iylae                   | 1                                  |
|    | Clostridium perfringens   | 1                                  | Micrococcus roseus                  | 1.                                 |
|    | Corynebacterium accolens  | 1                                  | Micrococcus varians                 | 1                                  |
| 10 | Corynebacterium aquaticum | . 1                                | Peptococcus niger                   | 1                                  |
|    | Corynebacterium bovis     | 1                                  | Peptostreptococcus anaerobius       | 1                                  |
|    | Corynebacterium cervicis  | 1                                  | Peptostreptococcus asaccharolyticus | 1                                  |
|    | Corynebacterium           | 6                                  | Staphylococcus aureus               | 10                                 |
| 15 | diphteriae                |                                    | •                                   |                                    |
|    | Corynebacterium           | 1                                  | Staphylococcus auricularis          | 1                                  |
|    | flavescens                |                                    |                                     |                                    |
|    | Corynebacterium           | 6                                  | Staphylococcus capitis              | 1                                  |
|    | genitalium                |                                    | subsp. <i>urealyticus</i>           |                                    |
| 20 | Corynebacterium jeikeium  | 1                                  | Staphylococcus cohnii               | 1                                  |
|    | Corynebacterium kutcheri  | 1                                  | Staphylococcus epidermidis          | 2                                  |
|    | Corynebacterium           | 1                                  | Staphylococcus                      | 2                                  |
|    | matruchotii               |                                    | haemolyticus                        |                                    |
|    | Corynebacterium           | 1                                  | Staphylococcus hominis              | 2                                  |
| 25 | minutissimum              |                                    |                                     |                                    |
|    | Corynebacterium           | 1                                  | Staphylococcus                      | 1                                  |
|    | mycetoides                |                                    | lugdunensis                         |                                    |
|    | Corynebacterium           | 1                                  | Staphylococcus                      | 3                                  |
|    | pseudodiphtheriticum      |                                    | saprophyticus                       |                                    |
| 30 | Corynebacterium           | 6                                  | Staphylococcus schleiferi           | 1                                  |
|    | pseudogenitalium          |                                    |                                     |                                    |
|    | Corynebacterium renale    | 1                                  | Staphylococcus sciuri               | 1                                  |
|    | Corynebacterium striatum  | 1 /                                | Staphylococcus simulans             | 1                                  |
|    | Corynebacterium ulcerans  | 1                                  | Staphylococcus warneri              | 1                                  |

|    | Bacterial species         | Number of | Bacterial species          | Number of |
|----|---------------------------|-----------|----------------------------|-----------|
|    |                           | reference |                            | reference |
|    |                           | strains   |                            | strains   |
|    |                           | testeda   |                            | tested    |
|    | Corynebacterium           | 1         | Staphylococcus xylosus     | 1         |
|    | urealyticum               |           |                            |           |
|    | Corynebacterium xerosis   | 1         | Streptococcus agalactiae   | 6         |
|    | Enterococcus avium        | 1         | Streptococcus anginosus    | 2         |
| 5  | Enterococcus              | 1         | Streptococcus bovis        | 2         |
|    | casseliflavus             |           |                            |           |
|    | Enterococcus cecorum      | 1         | Streptococcus constellatus | 1         |
|    | Enterococcus dispar       | 1         | Streptococcus crista       | 1         |
|    | Enterococcus durans       | 1         | Streptococcus dysgalactiae | 1         |
| 10 | Enterococcus faecalis     | 6         | Streptococcus equi         | 1         |
|    | Enterococcus faecium      | 3         | Streptococcus gordonii     | 1         |
|    | Enterococcus flavescens   | 1         | Group C Streptococci       | 1         |
|    | Enterococcus gallinarum   | 3         | Group D Streptococci       | 1         |
|    | Enterococcus hirae        | 1         | Group E Streptococci       | 1         |
| 15 | Enterococcus mundtii      | 1         | Group F Streptococci       | 1         |
|    | Enterococcus              | 1         | Group G Streptococci       | 1         |
|    | pseudoavium               | •         |                            |           |
|    | Enterococcus raffinosus   | 1         | Streptococcus intermedius  | 1         |
|    | Enterococcus              | 1         | Streptococcus mitis        | 2         |
| 20 | saccharolyticus           |           |                            |           |
|    | Enterococcus solitarius   | 1         | Streptococcus mutans       | 1         |
|    | Eubacterium lentum        | 1         | Streptococcus oralis       | 1         |
|    | Gemella haemolysans       | 1         | Streptococcus parasanguis  | 1         |
|    | Gemella morbillorum       | 1         | Streptococcus pneumoniae   | 6         |
| 25 | Lactobacillus acidophilus | 1         | Streptococcus pyogenes     | 3         |
|    | Listeria innocua          | 1         | Streptococcus salivarius   | 2         |
|    | Listeria ivanovii         | 1         | Streptococcus sanguis      | 2         |
|    | Listeria grayi            | 1         | Streptococcus sobrinus     | 1         |
|    | Listeria monocytogenes    | 3         | Streptococcus suis         | 1         |
| 30 | Listeria murrayi          | 1         | Streptococcus uberis       | 1         |
|    | Listeria seeligeri        | 1         | Streptococcus vestibularis | 1         |
|    | Listeria welshimeri       | 1         |                            |           |

Most reference strains were obtained from the American Type Culture Collection (ATCC). The other reference strains were obtained from (i) the Laboratoire de Santé Publique du Québec (LSPQ), (ii) the Center for Disease Control and Prevention (CDC) and (iii) the National Culture Type Collection (NCTC).

Table 6. Fungal species (12) used to test the specificity of PCR primers and DNA probes.

| Fungal species           | Number of reference strains tested <sup>a</sup> |  |
|--------------------------|---|--|
| Candida albicans         | 12  |  |
| Candida glabrata         | 1   |  |
| Candida guilliermondii   | 1   |  |
| Candida kefyr            | 3   |  |
| Candida krusei           | 2   |  |
| Candida lusitaniae       | 1   |  |
| Candida parapsilosis     | 2   |  |
| Candida tropicalis       | 3   |  |
| Rhodotorula glutinis     | 1   |  |
| Rhodotorula minuta       | . 1   |  |
| , Rhodotorula rubra      | 1   |  |
| Saccharomyces cerevisiae | · 1   |  |

Most reference strains were obtained from (i) the American Type Culture Collection
 (ATCC) and (ii) the Laboratoire de Santé Publique du Québec (LSPQ).

Table 7. PCR assays developed for several clinically important bacterial and fungal pathogens (continues on next page).

|    | Organism                         | Primer Pair <sup>a</sup> | Amplicon  | Ubiquity <sup>b</sup> | DNA amp  | lification from |
|----|----------------------------------|--------------------------|-----------|-----------------------|----------|-----------------|
|    |                                  | SEQ ID NO                | size (bp) |                       | culturec | specimensd      |
|    | Enterococcus faecium             | 1-2                      | 216       | 79/80                 | +        | +               |
| 5  | Listeria monocytogenes           | 3-4                      | 130       | 164/168°              | +        | +               |
|    | Neisseria meningitidis           | 5-6                      | 177       | 258/258               | +        | +               |
|    | Staphylococcus saprophyticus     | 7-8                      | 149       | 245/260               | +        | NT              |
| 10 | Streptococcus agalactiae         | 9-10                     | 154       | 29/29                 | +        | +               |
|    | Candida albicans                 | 11-12                    | 149       | 88/88                 | +        | NT              |
|    | Enterococcus                     | 13-14                    | 112       | 87/87                 | +        | NT              |
|    | spp. (11 species) <sup>f</sup>   |                          |           |                       |          |                 |
|    | Neisseria spp.                   | 15-16                    | 103       | 321/321               | +        | +               |
| 15 | (12 species) <sup>f</sup>        |                          |           |                       |          |                 |
|    | Staphylococcus spp.              | 17-18                    | 192       | 13/14                 | +        | NT              |
|    | (14 species)                     |                          |           |                       |          |                 |
|    | . •                              | 19-20                    | 221       | 13/14                 | +        | NT              |
|    | Streptococcus spp.               | 21-22                    | 153       | 210/214 <sup>9</sup>  | +        | +               |
| 20 | (22 species) <sup>f</sup>        |                          |           |                       |          |                 |
|    | Universal detection <sup>h</sup> | 23-24                    | 309       | 104/ 116 <sup>i</sup> | +        | +               |
|    | (95 species) <sup>i</sup>        |                          |           |                       |          |                 |

- All primer pairs are specific in PCR assays since no amplification was observed
   with DNA from the bacterial and fungal species other than the species of interest
   listed in Tables 4, 5 and 6.
  - b Ubiquity was tested by using reference strains as well as strains from throughout the world, which are representatite of the diversity within each target species or genus.
- <sup>c</sup> For all primer pairs, PCR amplifications performed directly from a standardized microbial suspension (MacFarland) or from a colony were all specific and ubiquitous.
  - d PCR assays performed directly from blood cultures, urine specimens or

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cerebrospinal fluid. NT, not tested.

- The four *L. monocytogenes* strains undetected are not clinical isolates. These strains were isolated from food and are not associated with a human infection.
- The bacterial species tested include all those clinically relevant for each genus (Tables 4 and 5). All of these species were efficiently amplified by their respective genus-specific PCR assay, except for the *Staphylococcus*-specific assay, which does not amplify *S. sciuri*.
  - The Streptococcus-specific PCR assay did not amplify 3 out of 9 strains of S. mutans and 1 out of 3 strains of S. salivarius.
- 10 h The primers selected for universal bacterial detection do not amplify DNA of non-bacterial origin, including human and other types of eukaryotic genomic DNA.
  - For the universal amplification, the 95 bacterial species tested represent the most clinically important bacterial species listed in Tables 4 and 5. The 12 strains not amplified are representatives of genera *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species).

Table 8. Target genes for the various genus-specific, species-specific and universal amplification assays.

| 20 | Microorganisms               | Gene    | Protein encoded                     |
|----|------------------------------|---------|-------------------------------------|
|    | Candida albicans             | tuf     | translation elongation factor EF-Tu |
|    | Enterococcus faecium         | ddl     | D-alanine:D-alanine ligase          |
|    | Listeria monocytogenes       | actA    | actin-assembly inducing protein     |
|    | Neisseria meningitidis       | omp     | outer membrane protein              |
| 25 | Streptococcus agalactiae     | cAMP    | cAMP factor                         |
|    | Staphylococcus saprophyticus | unknown | unknown                             |
|    | Enterococcus spp.            | tuf     | translation elongation factor EF-Tu |
|    | Neisseria spp.               | asd     | ASA-dehydrogenase                   |
| 30 | Staphylococcus spp.          | tuf     | translation elongation factor EF-Tu |
|    | Streptococcus spp.           | recA    | RecA protein                        |
|    | Universal detection          | tuf     | translation elongation factor EF-Tu |

Table 9. Antibiotic resistance genes selected for diagnostic purposes.

|   | Genes              | SEQ ID NOs          |                      | <b>Antibiotics</b> | Bacteria <sup>a</sup>                   |
|---|--------------------|---------------------|----------------------|--------------------|---|
|   | -                  | selected<br>primers | originating fragment | •                  |   |
| 5 | bla <sub>oxa</sub> | 49-50               | 110                  | β-lactams          | Enterobacteriaceae,<br>Pseudomonadaceae |
|   | blaZ               | 51-52               | 111                  | β-lactams          | Enterococcus spp.                       |
|   | aac6'-lla          | 61-64               | 112                  | Aminoglycosides    | Pseudomonadaceae                        |
|   | ermA               | 91-92               | 113                  | Macrolides         | Staphylococcus spp.                     |
| ) | ermB               | 93-94               | 114                  | Macrolides         | Staphylococcus spp.                     |
|   | ermC               | 95-96               | 115                  | Macrolides         | Staphylococcus spp.                     |
|   | vanB               | 71-74               | 116                  | Vancomycin         | Enterococcus spp.                       |
|   | vanC               | 75-76               | 117                  | Vancomycin         | Enterococcus spp.                       |
|   | aad(6')            | 173-174             | -                    | Streptomycin       | Enterococcus spp.                       |

<sup>&</sup>lt;sup>a</sup> Bacteria having high incidence for the specified antibiotic resistance genes. The presence of these antibiotic resistance genes in other bacteria is not excluded.

Table 10. Antibiotic resistance genes from our co-pending US (N.S. 08/526840) and PCT (PCT/CA/95/00528) patent applications for which we have selected PCR primer pairs.

| 5  | Genes              | SEQ ID NOs          | Antibiotics      | Bacteriaª              |
|----|--------------------|---------------------|------------------|------------------------|
|    |                    | of selected primers |                  |                        |
| ·. | bla <sub>tem</sub> | 37-40               | β-lactams        | Enterobacteriaceae,    |
|    |                    |                     |                  | Pseudomonadaceae,      |
|    |                    |                     |                  | Haemophilus spp.,      |
|    |                    |                     |                  | Neisseria spp.         |
|    | blamb              | 45-48               | β-lactams        | Haemophilus spp.,      |
|    |                    |                     |                  | Pasteurella spp.       |
| 10 | blashv             | 41-44               | β-lactams        | <i>Klebsiella</i> spp. |
|    |                    |                     |                  | and other              |
|    |                    |                     |                  | Enterobacteriaceae     |
|    | aadB               | 53-54               | Aminoglycosides  | Enterobacteriaceae,    |
|    | aacC1              | 55-56               |                  | Pseudomonadaceae       |
|    | aacC2              | 57-58               |                  |                        |
| 15 | aacC3              | 59-60               |                  |                        |
|    | aacA4              | 65-66               |                  |                        |
|    | mecA               | 97-98               | β-lactams        | Staphylococcus spp.    |
|    | vanA               | 67-70               | Vancomycin       | Enterococcus spp.      |
|    | satA               | 81-82               | Macrolides       | Enterococcus spp.      |
| 20 | aac(6')-aph(2")    | 83-86               | Aminoglycosides  | Enterococcus spp.,     |
|    |                    |                     |                  | Staphylococcus spp.    |
|    | vat                | 87-88               | Macrolides       | Staphylococcus spp.    |
|    | vga                | 89-90               | Macrolides       | Staphylococcus spp.    |
|    | msrA               | 77-80               | Erythromycin     | Staphylococcus spp.    |
|    | int                | 99-102              | β-lactams,       | Enterobacteriaceae,    |
| 25 |                    |                     | trimethoprim,    |                        |
|    | sul                | 103-106             | aminoglycosides, | Pseudomonadaceae       |
|    |                    |                     | antiseptic,      |                        |
|    |                    |                     | chloramphenicol  |                        |
|    |                    |                     |                  |                        |

Bacteria having high incidence for the specified antibiotic resistance genes. The presence of these antibiotic resistance genes in other bacteria is not excluded.

Table 11. Correlation between disk diffusion and PCR amplification of antibiotic resistance genes in *Staphylococcus* species<sup>a</sup>.

|    |              |                | <u></u> | Disk diffusion (Kirby-Bauer) <sup>b</sup> |              |           |
|----|--------------|----------------|---------|---|--------------|-----------|
| 5  | Antibiotic   | Phenotype      | PCR     | Resistant                                 | Intermediate | Sensitive |
|    | Penicillin   | blaZ           | +       | 165                                       | 0            | 0         |
|    |              |                | -       | 0   | 0            | 31        |
|    | Oxacillin    | mecA           | +       | 51  | 11           | 4         |
|    |              |                | -       | 2   | .0           | 128       |
| 10 | Gentamycin   | aac(6')aph(2") | +       | 24  | 18           | 6         |
|    |              |                | -       | 0   | 0            | 148       |
|    | Erythromycin | ermA           | +       | 15  | 0            | 0         |
|    |              | ermB           | +       | 0   | 0            | 0         |
|    |              | ermC           | +       | 43  | 0 -          | 0         |
| 15 | . •          | msrA           | +       | 4   | 0            | 0         |
|    |              |                | -       | 0   | 1            | 136       |

The Staphylococcus strains studied include S. aureus (82 strains), S. epidermidis (83 strains), S. hominis (2 strains), S. capitis (3 strains), S. haemolyticus (9 strains), S. simulans (12 strains) and S. warneri (5 strains), for a total of 196 strains.

<sup>&</sup>lt;sup>b</sup> Susceptibility testing was performed by the method of Kirby-Bauer according to the protocol reccommended by the National Committee of Clinical Laboratory Standards (NCCLS).

Table 12. Correlation between disk diffusion profiles and PCR amplification of antibiotic resistance genes in *Enterococcus* species\*.

|              |                 | _   | Disk diffusion | (Kirby-Bauer) <sup>b</sup> |
|--------------|-----------------|-----|----------------|----------------------------|
| Antibiotic   | Phenotype       | PCR | Resistant      | Sensitive                  |
|              | blaZ            | +   | 0              | 2                          |
| Ampicillin   |                 |     |                |                            |
|              |                 | -   | 1              | 30                         |
| Gentamycin   | aac(6')aph(2'') | +   | 51             | 1                          |
|              |                 | -   | 3              | 38                         |
| Streptomycin | aad(6')         | +   | 26             | 15                         |
|              |                 | -   | 6              | 27                         |
| Vancomycin   | vanA            | +   | 36             | 0                          |
|              | vanB            | +   | 26             | 0                          |
|              |                 | _   | 0              | 40                         |

The Enterococcus strains studied include E. faecalis (33 strains) and E. faecium (69 strains), for a total of 102 strains.

Susceptibility testing was performed by the method of Kirby-Bauer according to the protocol reccommended by the National Committee of Clinical Laboratory Standards (NCCLS).

Table 13. Origin of *tuf* sequences in the Sequence Listing (continues on next page).

| 1 | SEQ ID NO    | Bacterial or fungal species  | Source      |
|---|--------------|------------------------------|-------------|
| 5 | 118          | Abiotrophia adiacens         | This patent |
|   | 119          | Abiotrophia defectiva        | This patent |
|   | 120          | Candida albicans             | This patent |
|   | 121          | Candida glabrata             | This patent |
|   | 122          | Candida krusei               | This patent |
| כ | 123          | Candida parapsilosis         | This patent |
|   | 124          | Candida tropicalis           | This patent |
|   | 125          | Corynebacterium accolens     | This patent |
|   | 126          | Corynebacterium diphteriae   | This patent |
|   | 127          | Corynebacterium genitalium   | This patent |
| 5 | 128          | Corynebacterium jeikeium     | This patent |
|   | <b>129</b> , | Corynebacterium              | This patent |
|   |              | pseudotuberculosis           | · .         |
|   | 130          | Corynebacterium striatum     | This patent |
|   | 131          | Enterococcus avium           | This patent |
|   | 132          | Enterococcus faecalis        | This patent |
| ) | 133          | Enterococcus faecium         | This patent |
|   | 134          | Enterococcus gallinarum      | This patent |
|   | 135          | Gardnerella vaginalis        | This patent |
|   | 136          | Listeria innocua             | This patent |
|   | 137          | Listeria ivanovii            | This patent |
| 5 | i 138        | Listeria monocytogenes       | This patent |
|   | 139          | Listeria seeligeri           | This patent |
|   | 140          | Staphylococcus aureus        | This patent |
|   | 141          | Staphylococcus epidermidis   | This patent |
|   | 142          | Staphylococcus saprophyticus | This patent |
| 0 | 143          | Staphylococcus simulans      | This patent |
|   | 144          | Streptococcus agalactiae     | This patent |
|   | 145          | Streptococcus pneumoniae     | This patent |

|    | SEQ ID NO | Bacterial or fungal species | Source      |
|----|-----------|-----------------------------|-------------|
|    | 146       | Streptococcus salivarius    | This patent |
|    | 147       | Agrobacterium tumefaciens   | Database    |
|    | 148       | Bacillus subtilis           | Database    |
|    | 149       | Bacteroides fragilis        | Database    |
| 5  | 150       | Borrelia burgdorferi        | Database    |
|    | 151       | Brevibacterium linens       | Database    |
|    | 152       | Burkholderia cepacia        | Database    |
|    | 153       | Chlamydia trachomatis       | Database    |
|    | 154       | Escherichia coli            | Database    |
| 10 | 155       | Fibrobacter succinogenes    | Database    |
|    | 156       | Flavobacterium ferrugineum  | Database    |
|    | 157       | Haemophilus influenzae      | Database    |
|    | 158       | Helicobacter pylori         | Database    |
|    | 159       | Micrococcus luteus          | Database    |
| 15 | 160       | Mycobacterium tuberculosis  | Database    |
| ٠. | 161 ·     | Mycoplasma genitalium       | Database    |
|    | . 162     | Neisseria gonorrhoeae       | Database    |
|    | 163       | Rickettsia prowazekii       | Database    |
|    | 164       | Salmonella typhimurium      | Database    |
| 20 | 165       | Shewanella putida           | Database    |
|    | 166       | Stigmatella aurantiaca      | Database    |
|    | 167       | Streptococcus pyogenes      | Database    |
|    | 168       | Thiobacillus cuprinus       | Database    |
|    | 169       | Treponema pallidum          | Database    |
| 25 | 170       | Ureaplasma urealyticum      | Database    |
|    | 171       | Wolinella succinogenes      | Database    |

|    | Annex I:        | Strategy for   | for the selection         | from tuf          | sequences of t                     | the universal amplification | ion    |
|----|-----------------|--|---------------------------|-------------------|------------------------------------|-----------------------------|--------|
|    |                 | primers (conti   | (continues on pages       | 18 49 to 51)      | •                                  |                             |        |
|    |                 |  |                           |                   |                                    |                             | SEQ ID |
|    |                 | 491  |                           | 517776            |                                    | 802                         | NO     |
| 10 | Abiotrophia     | CAACTGTAAC TGG   | TGGTGTTGAA ATGTTCCAAATGGT | TCCAAATG          | T AATGCCTGGT GATAACGTAA            | GATAACGTAA                  | 118    |
|    | adiacens        |  |                           |                   |                                    |                             | •      |
|    | Abiotrophia     | CTACCGTTAC CGC   | CGGTGTTGAA ATGTTCCAAATGGT | ICCAAAIG          | TATGCCAGGC GACAACGTAC              | GACAACGIAC                  | 119    |
|    | defectiva       |  |                           |                   |                                    |                             |        |
|    | Agrobacterium   | CGACTGTTAC CGC   | CGGCGTTGAA AIGIICCAAAIGGI | ICCAAAIG          | TATGCCTGGC GACAACGTCA              | GACAACGICA                  | 147    |
| _  | tumefaciens     |  |                           |                   |                                    |                             |        |
|    | Bacillus        | CAACTGITAC AGGIGITGAA AIGIICCAAAIGGI                       | TGTTGAA ATGT              | ICCAAAIG          | TATGCCTGGA GATAACACTG              | GATAACACTG                  | 148    |
|    | subtilis        |  |                           |                   |                                    |                             |        |
|    | Bacteroides     | CAGTIGIAAC AGG   | AGGIGITGAA AIGIICCAAAIGGI | ICCAAAIG          | T AATGCCGGGT GATAACGTAA            | GATAACGIAA                  | 149    |
|    | fragilis        |  |                           |                   |                                    |                             |        |
|    | Borrelia        | CTACTGTTAC IGG   | IGGIGITGAA AIGIICCAAAIGGI | ICCAAAIGG         | TATGCCTGGT                         | GATAAIGI                    | 150    |
|    | burgdorferi     |  |                           |                   |                                    |                             |        |
|    | Brevibacterium  | CGACTGTCAC CGCTATCGAG                                      |                           | ICCAGAIGG         | ATGITC: AGATGGI CATGCCCGGC GACACCA | GACACCA                     | 151    |
|    | linens          |  |                           | : 1.4<br>::<br>:: |                                    |                             |        |
|    | Burkholderia    | CGACCTGCAC GGGCGTTGAA ATGTTCCAAATGGT                       | CGTTGAA AIGT              | rccaa <u>arg</u>  | CATGCCGGGC                         | GACAACGTGT                  | 152    |
|    | cepacia         |  |                           |                   |                                    |                             |        |
|    | Chlamydia       | CGATIGITAC IGGGGIIGAA                                      | GGTIGAA ATGTICA           | CAAGATGGT         | T CATGCCTGGG GATAACGTTG            | GATAACGTTG                  | 153    |
|    | trachomatis     |  |                           | ,                 |                                    |                             |        |
|    | Corynebacterium | CCACCGTTAC CGGTATCGAG ATGTTCCAGATGGT CATGCCTGGC GACAACGTCG | TAICGAG AIGT              | ICCAG <u>AIG</u>  | T CATGCCTGGC                       | GACAACGICG                  | 126    |
|    | diphteriae      |  |                           |                   |                                    |                             |        |
|    |                 |  |                           |                   |                                    |                             |        |

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|    | Corynebacterium | CCACCGTTAC CTCC  | CTCCATCGAG        | AIGIICA | AGATGGT   | TATGCCGGGC            | GACAACGTTG            | 127 |
|----|-----------------|------------------|-------------------|---------|-----------|-----------------------|-----------------------|-----|
|    | genitalium      |                  |                   |         |           |                       |                       |     |
|    | Corynebacterium | CCACCGTTAC CTCC  | <u>CTCCATCGAG</u> | AIGIICA | AGATGGT   | TATGCCGGGC            | GACAACGTTG            | 128 |
|    | jeikeium        |                  |                   |         |           | 74.                   |                       |     |
| 10 | Enterococcus    | CAACYGTTAC AGGTO | AGGTGTTGAA        | AIGIICC | AAATGGT   | AATGCCTGGT            | GATAACGITG            | 132 |
|    | faecalis        |                  |                   |         |           |                       |                       |     |
|    | Enterococcus    | CAACAGTTAC TGGTG | TGGTGTTGAA        | AIGTICC | . AAATGGT | CATGCCCGGT            | GACAACGI              | 133 |
|    | faecium         |                  |                   |         |           |                       |                       |     |
|    | Escherichia     | CTACCTGTAC TGGCC | TGGCGTTGAA        | ATGTTCC | AGATGGT   | AATGCCGGGC            | GACAACAICA            | 154 |
| _  | coli            |                  |                   |         |           |                       |                       |     |
|    | Fibrobacter     | ACGTCATCAC CGGTO | CGGTGTTGAA        | AIGIICC | AAATGGT   | TACTCCGGGT            | GACACGGICA            | 155 |
|    | succinogenes    |                  |                   |         |           |                       |                       |     |
|    | Flavobacterium  | CTACCGTTAC AGGT  | AGGTGTTGAG        | ATGTTCC | AAATGGT   | TATGCCTGGT            | TATGCCTGGT GATAACACCA | 156 |
|    | ferrugineum     |                  |                   |         |           |                       |                       |     |
| 10 | Gardnerella     | CCACCGTCAC CTCL  | CTCIAICGAG        | ACCITCC | AAATGGT   | TCAGCCAGGC            | GATCACGCAA            | 135 |
|    | vaginalis       |                  |                   |         |           |                       |                       |     |
|    | Haemophilus     | CTACTGTAAC GGGTG | GGGTGTTGAA        | ATGTTCC | . AAATGGT | AATGCCAGGC            | GATACATCA             | 157 |
|    | influenzae      |                  | ·                 |         |           |                       |                       |     |
|    | Helicobacter    | CGACTGTAAC CGGTC | CGGTGTAGAA        | ATGTTTA | AAATGGT   | TATGCCTGGC            | GATAAIGIGA            | 158 |
|    | pylori          |                  |                   |         |           |                       |                       |     |
|    | Listeria        | TAGTAGTAAC TGGAG | TGGAGTAGAA        | ATGTTCC | AAATGGT   | AAYGCCTGGT            | GATACATTG             | 138 |
|    | monocytogenes   |                  |                   |         |           |                       |                       |     |
|    | Micrococcus     | CCACTGTCAC CGGC  | CGGCATCGAG        | ATGTICC | AGATGGT   | CATGCCCGGC            | GACAACACG             | 159 |
|    | luteus          |                  |                   |         |           |                       |                       |     |
| 10 | Mycobacterium   | CCACCGTCAC CGGTG | CGGTGTGGAG        | AIGII   | AGATGGT   | GATGCCCGGT GACAACACCA | GACAACA               | 160 |

|         | Mycoplasma      | CAGTIGITAC IGGAA                      | LTGAA  | AIGIICA. | IGGAAIIGAA AIGIICAAAAIGGI | TCLACCTGGT            | GATAATGCTT            | 161 |
|---------|-----------------|---------------------------------------|--------|----------|---------------------------|-----------------------|-----------------------|-----|
|         | genitalium      |                                       |        |          |                           |                       |                       |     |
|         | Neisseria       | CCACCTGTAC CGGCGTTGAA ATGTTCC AAATGGT | LTGAA  | ATGTTCC. | AAATGGT                   | AATGCCGGGT            | AATGCCGGGT GAGAACGTAA | 162 |
|         | gonorrhoeae     |                                       |        |          |                           |                       |                       |     |
| Ŋ       | Rickettsia      | CGACTIGIAC AGGIGIAGAA                 |        | ATGTTCA. | ATGIICAAGAIGGI            | TATGCCTGGA            | GATAATGCTA            | 163 |
|         | prowazekii      |                                       |        |          |                           |                       |                       |     |
|         | Salmonella      | CTACCTGTAC IGGCGIIGAA AIGIICCAGAIGGI  | LTGAA  | AIGIICC. | AGATGGT                   |                       | AATGCCGGGC GACAACAICA | 164 |
|         | typhimurium     |                                       |        |          |                           |                       |                       |     |
|         | Shewanella      | CAACGTGTAC TGGTGTAGAA                 |        | ATGTTCC. | ATGTTCCAGATGGT            |                       | AATGCCAGGC GATAACATCA | 165 |
| ,<br>10 | putida          |                                       |        |          |                           |                       |                       |     |
|         | Sti $g$ matella | CGGTCATCAC GGGGGTGGAG ATGTTCCAGATGGT  | FGGAG  | AIGIICC. | AGATGGT                   | GATGCCGGGA            | GACAACATCG            | 166 |
|         | aurantiaca      |                                       |        |          |                           |                       |                       |     |
| ٠       | Staphylococcus  | CAACTGTTAC AGGTGTTGAA                 |        | AIGIICC. | ATGTTCCAAATGGT            | AATGCCTGGT            | GATAACGTTG            | 140 |
|         | aureus          |                                       |        |          |                           |                       |                       |     |
| 15      | Staphylococcus  | CAACTGTTAC TGGTGTAGAA ATGTTCCAAATGGT  | AGAA A | TGTTCC   |                           | TATGCCTGGC GACAACGTTG | GACAACGITG            | 141 |
|         | epidermidis     |                                       |        |          |                           |                       |                       |     |
|         | Streptococcus   | CAGTIGITAC IGGIGITGAA                 |        | AIGIICC. | AAATGGT                   | TATGCCTGGT            | TATGCCTGGT GATAACGTTA | 144 |
|         | agalactiae      |                                       |        |          |                           |                       |                       |     |
|         | Streptococcus   | CAGTIGITAC IGGIGITGAA                 |        | ATGIICC. | ATGITCCAAAIGGI            | AATGCCTGGT            | GATAACGIGA            | 145 |
| 20      | pneumoniae      |                                       |        |          |                           |                       |                       |     |
|         | Streptococcus   | CTGTTGTTAC TGGTGTTGAA                 |        | AIGIICC. | AAATGGT                   | TATGCCTGGT            | GATAACGTGA            | 167 |
|         | pyogenes        |                                       |        |          |                           |                       |                       |     |
|         | Thiobacillus    | CCACCTGCAC CGGCGTGGAA                 |        | ATGTTCA. | ATGTICAAAATGGI            | CATGCCCGGC            | GATAATGIGA            | 168 |
|         | cuprinus        |                                       |        |          |                           |                       |                       |     |
| 25      | Treponema       | CAGTGGTTAC TGGCATTGAG                 |        | ATGITTA. | ACATGGT                   | GAAGCCGGGG            | GATAACACCA            | 169 |
|         |                 |                                       |        |          |                           |                       |                       |     |

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Cor

or G; "R" stands for A or G; "Y" stands for

sequence.

complement of the above tuf

This sequence is the reverse

"K" stands for

degenerated.

| Ureaplasma       | CTGTTGTTAC AGGAATTGAA ATGTTTAATTTGGT TATGCCAGGT GATGACGTTG  | 170    |
|------------------|---|--------|
| urealyticum      |   |        |
| Wolinella        | CAACCGTAAC IGGCGTIGAG AIGIICCAGAIGGI TAIGCCIGGI GACAACGIIA  | 171    |
| succinogenes     |   |        |
| Candida          | GTGTIAC <u>cac tgaagtcaar</u> tccgitgagraat <u>i ggaagaaat</u> cc <u>aaaa</u> at <u>i</u> cg                  | 120    |
| albicans         |   |        |
| Schizo-          | GTGTCACTAC CGAAGICAAG TCTGITGAGAAGAI IGAGGAGICC CCIAAGIITG  |        |
| saccharomyces po | pombe   |        |
| Human            | TG <u>acaggca</u> t <u>Igagaig</u> ttc cacaagaag <u>aaggag</u> c <u>i</u> tg <u>cc</u> atg cc <u>c</u> gggagg |        |
| Selecteda        | ACIKKIAC IGGIGTIGAR ATGTT ATGGT IATGCCIGGI GAIAAYRT   |        |
| equencesª        |   |        |
|                  |   |        |
| Selected         | SEQ ID NO:23  |        |
| universal        |   |        |
| primer           | ACIKKIAC IGGIGTIGAR ATGTT AYRTT ITCICCIGGC ATIACCAT   |        |
| sequences:       |   |        |
|                  |   |        |
| The sequence num | The sequence numbering refers to the $E.$ $coli$ $tuf$ gene fragment. Underlined nucleotides                  | es are |
| identical to the | to the selected sequence or match that sequence.  |        |
| a "I" stands     | stands for inosine which is a nucleotide analog that can bind to any of the                                   | e four |
| nucleotides      | es A, C, G or T. "K", "R" and "Y" designate nucleotide positions which  | h are  |

10

|    | Annex II:       | Strategy for the selection from tuf sequences of the | ne amplification primers specific for     |   |
|----|-----------------|--|---|---|
|    |                 | the genus Enterococcus (continues on pages 53 and 5  | 54).                                      |   |
|    |                 | 314 348 401  | 435 SEQ                                   |   |
|    |                 |  | ON GI                                     | _ |
| ស  | Bacillus        | CGCGACACTG AAAACCATT CATGATGCCA GTTGACGCGG AG        | ACAAGTTAAA GICGGIGACG AAGTIGAAAT 148      |   |
|    | subtilis        |  |   |   |
|    | Bacteroides     | CGCGA <u>IGTIG ATAAACCTIT CTIGAIG</u> CCG GTAGAACTGG | TGTTAICCAT GIAGGIGATG AAAICGAAAT 149      |   |
|    | fragilis        |  |   |   |
|    | Burkholderia    | CGTGCAGTIG ACGCGCGII CCIGAIGCCG GTGGA CGCGG          | CATC <u>GIGAAG GICGGCGAAG AAAI</u> CGAAAT |   |
| 10 | cepacia         |  |   |   |
|    | Chlamydia       | AGAGAATIG ACAAGCCTIT CTIAAIGCCT ATTGACGTGG A         | AATTGTTAAA GTTTCCGATA AAGTTCAGTT 153      |   |
|    | trachomatis     | S  |   |   |
|    | Corynebacterium | CGTGAGACCG ACAAGCCATT CCTCATGCCT ATCGACGTGG          | CTCCCIGAAG GICAACGAGG ACGICGAGAT 126      |   |
|    | diphteriae      |  |   |   |
| 15 | Enterococcus    | CGTGATACTG ACAAACCATT CATGATGCCA GTCGACGTGG          | ACAAGTTCGC GTTGGTGACG AAGTTGAAAT 131      |   |
|    | avium           |  |   |   |
|    | Enterococcus    | CGTGATACTG ACAAACCATT CATGATGCCA GTCGACGTGG          | TGAAGTICGC GTIGGIGACG AAGIIGAAAT 132      |   |
|    | faecalis        |  |   |   |
|    | Enterococcus    | CGTGACAACG ACAAACCATT CATGATGCCA GTTGACGTGG          | ACAAGTICGC GIIGGIGACG AAGIIGAAGT          |   |
| 20 | faecium         |  |   |   |
|    | Enterococcus    | CGTGATACTG ACAAACCATT CATGATGCCA GTCGACGTGG          | ACAAGTICGC GTIGGIGAIG AAGIAGAAAT 134      |   |
|    | gallinarum      |  |   |   |
|    | Escherichia     | CGTGCGATIG ACAAGCCGTI CCIGCIGCCG ATCGACGCGG          | TATCAICAAA GIIGGIGAAG AAGIIGAAAT 154      |   |
|    | 100             |  |   |   |

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|    | Gardnerella    | CACGA <u>ICTIG ACAAGCCATI</u> CT <u>IGAIG</u> CCA ATCGACGTGG TAAGC <u>ICC</u> CA A <u>ICAACACCC CAGTI</u> GAGAT 135 | ហ         |
|----|----------------|---|-----------|
|    | vaginalis      |   |           |
|    | Haemophilus    | CGTGCGATIG ACCAACCGII CCITCITCCA ATCGACGAGG TATTAICCGT ACAGGIGATG AAGIAGAAAT 157                                    | 7         |
|    | influenzae     |   |           |
| Ŋ  | Helicobacter   | AGAGAC <u>ACTG AAAAACTIT CTIGATG</u> CCG GTTGAAGAGG CGTG <u>GIGAAA GIAGGCGA</u> T <u>G AAGI</u> GGAAAT 158          | <b>60</b> |
|    | pylori         |   |           |
|    | Listeria       | CGTGA <u>TACTG</u> ACAAACCATT CATGATGCCA GTTGACGTGG ACAAGTTAAA GTTGGTGACG AAGTAGAAGT 138                            | ω         |
|    | monocytogenes  |   |           |
|    | Micrococcus    | CGCGACAAGG ACAAGCCGIT CCTGATGCCG ATCGACGCGG CACCCTGAAG ATCAACTCCG AGGTCGAGAT 159                                    | 9         |
| 10 | 10 luteus      |   |           |
|    | Mycobacterium  | CGCGAGACCG ACAAGCCGTT CCTGATGCCG GTCGACGCGG CGTGATCAAC GTGAACGAGG AAGTTGAGAT 160                                    | 0         |
|    | tuberculosis   |   |           |
|    | Mycoplasma     | CGTGAAGTAG ATAAACCTIT CTTATTAGCA ATTGAAGAGG TGAACICAAA GTAGGICAAG AAGTIGAAAT 161                                    | Ħ         |
|    | genitalium     |   |           |
| 15 | Neisseria      | CGTGCCGTGG ACAAACCATT CCTGCTGCCT ATCGACGAGG TATCATCCAC GTTGGTGACG AGATTGAAAT 162                                    | 7         |
|    | gonorrhoeae    |   |           |
|    | Salmonella     | CGTGCGATIG ACAAGCCGTI CCIGCIGCCG ATCGACGCGG TATCAICAAA GIGGGCGAAG AAGIIGAAAT 164                                    | 4         |
|    | typhimurium    |   |           |
|    | Shewanella     | CGTGACATCG ATAAGCCGTT CCTACTGCCA ATCGACGTGG TATTGTACGC GTAGGCGACG AAGTTGAAAT 165                                    | ß         |
| 20 | putida         |   |           |
|    | Staphylococcus | rga   | 0         |
|    | aureus         |   |           |
|    | Staphylococcus | CGTGA <u>TICIG ACAAACCATI CAIGAIG</u> CCA GTTGACGTGG TCAAA <u>ICAAA GIWGGTGAAG AAGTI</u> GAAAT 141                  | -         |
|    | epidermidis    |   |           |
| 25 | Staphylococcus | CGTGA <u>TICTG ACAAACCATT CATGATG</u> CCA GTTGACGTGG TCAAA <u>ICAAA GTCGGTGAAG AAAI</u> CGARAT                      | 73        |
|    | saprophyticus  |   |           |
|    |                |   |           |

ACITCITCCA GITGA...CGTGG TACTGITCGT GICAACGACG AAGIIGAAAT

CGTGATACTG ACAAACCTTT

| agalactiae       |                       |   |                       | ,                  |           |                   |   |  |     |
|------------------|-----------------------|---|-----------------------|--------------------|-----------|-------------------|---|--|-----|
| Streptococcus    | CGTGACACTG            | CGTGACACTG ACAAACCATT GCTTCTTCCA GTCGACGTGG | GCTICTICCA            | GTCGA              |           | TC <u>GTT</u> AAA | GICAACGACG  | TATC <u>GTT</u> AAA <u>GT</u> CAAC <u>GACG AAAT</u> CGAAAT | 145 |
| pneumoniae       |                       |   |                       |                    |           |                   |   |  |     |
| Streptococcus    | CGCGACACTG            | CGCGACACIG ACAAACCATT GCITCITCCA GTCGACGTGG | GCTTCTTCCA            | GTCGA              |           | CTGTTCGT          | TACT <u>GIICG</u> T <u>GI</u> CAACGACG ABAICGAAAT | AAAICGAAAT   | 167 |
| pyogenes         |                       |   |                       |                    |           |                   |   |  |     |
| Ureaplasma       | CGTAGTACTG ACAAACCATT |   | CTTATTAGCA ATTGACGTGG | ATTGA              |           | TATTAAAA          | <u>GTTAATGATG</u>                                 | tgtat <u>taaaa gttaatga</u> t <u>g aggtt</u> gaaat         | 170 |
| urealyticum      |                       |   |                       |                    |           |                   |   |  |     |
| Selected         | TACTG                 | TACTG ACAAACCATT                            | CATGATG               |                    |           | GTTCGC            | GTTGGTGACG AAGTT                                  | AAGTT  |     |
| sednences (      |                       |   |                       |                    |           |                   |   |  |     |
|                  |                       |   |                       |                    |           |                   |   |  |     |
| Selected         | S                     | SEQ ID NO: 1                                | 13                    |                    |           | SE                | SEQ ID NO: 14ª                                    | æ.   |     |
| genus-specific   |                       |   |                       |                    | ٠.        |                   |   |  |     |
| primer           | TACTG                 | TACTG ACAAACCATT CATGATG                    | CATGATG               |                    |           | AACTIC            | AACTTC GTCACCAACG CGAAC                           | CGAAC  |     |
| s sequences:     |                       |   |                       |                    |           |                   |   |  |     |
|                  |                       |   |                       | ,                  | ,         |                   |   |  |     |
| The sequence nur | numbering refers      | is to the $E$ .                             | faecalis              | tu <u>f</u> , gene | fragment. |                   | Underlined nucleotides                            | cides are  |     |

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sequence. match that о Н selected sequence the identical

tuf sequence. above the complement of This 20

genus has this species; The above primers also amplify tuf sequences from Abiotrophia 16S rRNA analysis. been related to the Enterococcus genus by NOTE:

|          | Annex III:      | Strategy for the selection | on from tuf sequences of the amplification primers specific | for    |
|----------|-----------------|----------------------------|---|--------|
|          |                 | the genus Staphylococcus   | (continues on pages 56 and 57).                             |        |
|          |                 | 385                        | 420579  | SEQ ID |
|          |                 |                            |   | NO     |
| <b>*</b> | 5 Bacillus      | TGGCCGTGTA GAACGCGGAC      | AC AAGITAAAGT CGGTTG CTAAACCAGG TACAATCACT CCACACAGCA       | 148    |
|          | subtilis        |                            |   |        |
|          | Bacteroides     | AGGTCGTATC GAAACTGGTG      | IG TTAICCATGT AGGTIT GTAAACCGGG ICAGAIIAAA CCTCACTCTA       | 149    |
|          | fragilis        |                            |   |        |
|          | Burkholderia    | GGGTCGTGTC GAGCGCGCA       | CA TCGIGAAGGT CGGTGG CGAAGCCGGG TTCGATCACG CCGCACACGC       | 152    |
| 10       | O cepacia       |                            |   |        |
|          | Chlamydia       | TGGACGTATT GAGCGTGGAA      | AA TTGITAAAGT TTCTTT GCTTGCCAAA CAGIGITAAA CCTCAIA          | 153    |
|          | trachomatis     |                            |   |        |
|          | Corynebacterium | n CGGCCGTGTT GAGCGTGGCT    | CT CCCIGAAGGT CAATTG TTAAGCCAGG CGCTTACACC CCTCACACCG       | 126    |
|          | diphteriae      |                            |   |        |
| 15       | 5 Enterococcus  | AGGACGTGTT GAACGTGGTG      | EG AAGITCGCGT TGGTAG CTAAACCAGC TACAATCACT CCACACACA        | 132    |
|          | faecalis        |                            |   |        |
|          | Enterococcus    | AGGTCGTGTT GAACGTGGAC      | AC AAGITCGCGT TGGTAG CTAAACCAGG TACAATCACA CCTCRIACAA       | 133    |
|          | faecium         |                            |   |        |
|          | Escherichia     | CGGTCTTA GAACGCGGTA        | LA TCATCAAAGT TGGTGG CTAAGCCGGG CACCATCAAG CCGCACACCA       | 154    |
| 20       | o coli          |                            |   |        |
|          | Gardnerella     | CGGTCGTGTT GAGCGTGGTA      | EA AGCICCCAAT CAATGG CTGCICCAGG IICIGIGACT CCACACACCA       | 135    |
|          | vaqinalis       |                            |   |        |

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|    | <i>Haemophilus</i> | AGGTCGTGTA GAACGAGGTA TTATCCGTAC AGGTAG CGAAACCAGG TTCAATCACA CCACACTG 157                             | 57 |
|----|--------------------|--|----|
|    | influenzae         |  |    |
|    | Helicobacter       | AGGTAGGATT GAAAGAGGCG TGGTGAAAGT AGGTAT GCAAACCAGG TTCTATCACT CCGCACAAGA 158                           | 28 |
|    | pylori             |  |    |
| 2  | Listeria           | TGGA <u>CGTGTT GAACGTGGAC AAGTTAAA</u> GT TGGTAG CTAAA <u>CCAGG TTCGATTAC</u> T <u>CCACACA</u> CTA 138 | 38 |
|    | monocytogenes      |  |    |
|    | Micrococcus        | CGGT <u>CG</u> CGCC GAGCGCGCA CCCTGAAGAT CAATGG TGGAGCCGGG CTCCATCACC CCGCACACCA 159                   | 59 |
|    | luteus             |  |    |
|    | Mycobacterium      | CGGACGIGIG GAGCGCGCG TGAICAACGI GAATCA CCAAGCCCGG CACCACCACG CCGCACACAAAAAAAA                          | 09 |
| 10 | tuberculosis       |  |    |
|    | Mycoplasma         | AGGAAGAGTT GAAAGAGGTG AACTCAAAGT AGGTAG CAAAACCAGG CTCTATTAAA CCGCACAAGA 161                           | 61 |
|    | genitalium         |  |    |
|    | Neisseria          | CGGCCGTGTA GAGCGAGGTA TCATCCACGT TGGTGG CCAAACGGGG TACTATCACT CCTCACACCA 162                           | 62 |
|    | gonorrhoeae        |  |    |
| 15 | Salmonella         | CGGTCGTGTA GAGCGCGCGGTA TCATCAAAGT GGGTGG CTAAGCCGGG CACCATCAAG CCGCACACCA                             | 64 |
|    | typhimurium        |  |    |
|    | Shewanella         | AGGT <u>CGTGTT GAGCGTGATA TTGTACGCGT AGGTAG CGAAGCCAGG TTCAATCAAC CCACACA</u> CTA 165                  | 65 |
|    | putida             |  |    |
|    | Staphylococcus     | AGGCCGTGTT GAACGTGGTC AAATCAAAGT TGGTAG CTGCTCCTGG TTCAATTACA CCACATACTG 140                           | 40 |
| 20 | aureus             |  |    |
|    | Staphylococcus     | AGGCCGTGTT GAACGTGGTC AAATCAAAGT WGGTAG CTGCTCCTGG TTCTATTACA CCACACACAA 141                           | 41 |
|    | epidermidis        |  |    |
|    | Staphylococcus     | AGGCCGTGTT GAACGTGGTC AAATCAAAGT CGGTAG CTGCTCCTGG TACTATCACA CCACATACAA 142                           | 42 |
|    | saprophyticus      |  |    |
| 25 | Staphylococcus     | AGGCCGTGTT GAACGTGGTC AAATCAAAGT CGGTAG CAGCTCCTGG CTCTATTACT CCACACACAA 143                           | 43 |
|    | simulans           |  |    |
|    |                    |  |    |

TRIGIGGI GIRATWGWRC CAGGAGC

CCGTGTT GAACGTGGTC AAATCAAA

genus-specific

primer

| #<br>#<br>-i                        |            | 145                                  |            | 170                                   |             |                             |           |                            |
|-------------------------------------|------------|--------------------------------------|------------|---------------------------------------|-------------|-----------------------------|-----------|----------------------------|
| HITUHANA ANTINUTTE BOUTANNIA        |            | CTAAACCAGG TTCAATCAAC CCACACACTA     |            | TAAAACCAGG ATCAATTAAA CCTCACCGTA      |             | GCTCCTGG XWCWATYACA CCACAYA |           | SEQ ID NO: 18 <sup>b</sup> |
| 4471                                |            | TTCAA                                |            | ATCAA                                 |             | XWCWA                       |           | SEQ ID                     |
| SSH TAWWY 1                         |            |                                      |            |                                       |             | GCTCCTGG                    |           | OJ.                        |
| 5 T T                               |            | AATCG                                |            | AATTG                                 |             |                             |           |                            |
| AGGALGAAL GACCAAGGAA CIGAICIGI CAA. |            | AGGACGIAIC GACCGIGGIA TCGITAAAGT CAA |            | TGGACGTGTT GAACGTGGTG TATTAAAAGT TAA. |             | AAATCAAA                    |           | 17                         |
| サイラのイダンンなり                          |            | GACCGTGGTA                           |            | GAACGTGGTG                            |             | CCGTGTT GAACGTGGTC AAATCAAA |           | SEQ ID NO: 17              |
| ノマルマラブルりりゃ                          |            | agga <u>cgtat</u> c                  |            | TGGACGTGTT                            |             | CCGTGT                      |           |                            |
| snoocoodeins                        | agalactiae | Streptococcus                        | pneumoniae | 5 Ureaplasma                          | urealyticum | Selected                    | sequences | 10 Selected                |
|                                     |            |                                      |            |                                       |             |                             |           |                            |

aureus tuf gene fragment. Underlined nucleotides are identical sequence. The sequence numbering refers to the S to the selected sequence or match that sequences\*:

"R" stands for "R", "W" and "Y" designate nucleotide positions which are degenerated. for A or T; "Y", for C or

ö

This sequence is the reverse complement of the above tuf sequence

| aplification primers specific for t |            |
|-------------------------------------|------------|
| the ampl                            | and 60).   |
| of                                  | 59 a       |
| sednences                           | on pages   |
| tuf                                 |            |
| from                                | (continues |
| ne selection                        | albicans   |
| for the                             | Candida    |
|                                     |            |
| Strategy                            | species    |
| Annex IV:                           |            |

|    |                     | 58                    |                              | 90   | 181   |                           |                                      | 213 SEQ            | ID NO |
|----|---------------------|-----------------------|------------------------------|------|-------|---------------------------|--------------------------------------|--------------------|-------|
|    | Candida             | CGTCAAGAAG GTTGGTTACA | GTTGGTTACA ACCCAAAGAC        | TGT  | CAA   | ATCCGGTAAA                | CAA AICCGGIAAA GITACIGGIA AGACCIIGIT | AGACCTTGTT         | 120   |
|    | albicans            |                       |                              |      |       |                           |                                      |                    |       |
|    | Candida             | CATCAAGAAG GICGGITACA | ACCCAAAGAC                   | TGT  | . CAA | GGCTGGTGTC                | GICAAGGGIA                           | AGAYCTTGTT         | 121   |
| ß  | glabrata            |                       |                              |      |       |                           |                                      |                    |       |
|    | Candida             | CATCAAGAAG GTTGGTTACA | GTTGGTTACA ACCCAAAGAC        | TGT  | .CAA  | CAA GGCAGGIGTT            | GTTAAGGGTA                           | AGACCTTATT         | 122   |
|    | krusei              |                       | 2                            |      |       |                           |                                      |                    |       |
|    | Candida             | CGTCAAGAAG GTTGGTTACA | ACCCTAAAGC                   | TGT  | . TAA | AGCTGGTAAG                | GTTACCGGTA                           | AGACCTIGIT         | 123   |
|    | parapsilosis        |                       |                              |      |       |                           |                                      |                    |       |
| 10 | Candida             | CGTCAAGAAG GTTGGTTACA | ACCCTAAGGC                   | TGT  | CAA   | GGCTGGTAAG                | GTTACCGGTA AGACTTTGTT                | AGACTTTGTT         | 124   |
|    | tropicalis          |                       |                              |      |       |                           |                                      |                    |       |
|    | Schizo-             | CATCAAGAAG GICGGIITCA | ACCCCAAGAC                   | CGT  | CAA.  | . CAA GG <u>CTGGT</u> GTC | GICAAGGGIA                           | AGACTCTTTT         |       |
|    | saccharomyces pombe | <b>U</b>              |                              |      |       |                           |                                      |                    |       |
|    | Human               | GGAGATCCGG GAGCTGCTCA | CCGAGITIGG                   | CIA  | GTT   | AGGCTGAAG                 | TCIGTGCAGA                           | <u>ag</u> ctactgga |       |
| 15 | Chlamydia           | GGAGCTGCGC GAGCTGCTCA | GCAAGTACGG                   | CTT  | CAA   | AIG                       | IATICIGG                             | <u>ag</u> ctgatgaa | 153   |
|    | trachomatis         |                       |                              |      |       |                           |                                      |                    |       |
|    | Corynebacterium     | GGAGATCCRT GAGCTGCTCG | CTGAGCAGGA                   | TTA  | GAA   | GTGGACCCAG                | TCCATCATCG                           | <u>Acctea</u> tgea | 126   |
|    | diphteriae          |                       |                              |      |       |                           |                                      |                    |       |
|    | Enterococcus        | GGAAGTTCGT GACTTAITAT | CAGA <u>a</u> t <u>a</u> cga | TTT. | •     | TGAAGAA                   | .TGAAGAA AAAATCTTAG                  | AATTAATGGC         | 132   |
| 20 | faecalis            |                       |                              |      |       |                           |                                      |                    |       |
|    | Escherichia         | GGAAGTTCGT GAACTICTGT | CTCAGTACGA                   | CTT  | •     | GGGAAGCG                  | AAAATCCTGG                           | <b>AACTGG</b> CTGG | 154   |
|    | coli                |                       |                              |      |       |                           |                                      |                    |       |

- 59 -

|    | Flavobacterium | CGAGGTTCGC          | GAAGAACTGA            | CTAAACGCGG          | TTT    | GGGTTAAA           | GAAATTGAAA                   | ACCTGATGGA         | 156 |
|----|----------------|---------------------|-----------------------|---------------------|--------|--------------------|------------------------------|--------------------|-----|
|    | ferrugineum    |                     | ·                     |                     |        |                    |                              |                    |     |
|    | Gardnerella    | AGAGGTCCGT          | GACCTCCTCG            | AAGAAAACGG          | CTTCAA | <u>Gigggtaga</u> g | GIGGGTAGAG ACCGTCAAGG        | <u>aactca</u> tgaa | 135 |
|    | vaginalis      |                     |                       |                     |        |                    |                              |                    |     |
| Ŋ  | Haemophilus    | GGAAGTTCGT          | GAACTICTAT            | CT <u>Caa</u> tatga | CTT    | GGGAAGAA           | GGGAAGAA AAAATCCTIG          | <u>ag</u> ttagcaaa | 157 |
|    | influenzae     |                     |                       |                     |        |                    |                              |                    |     |
|    | Listeria       | GGA <u>AA</u> TTCGT | GAICTAITAA            | CTGAATATGA          | ATT    | GGGAAGCT           | AAAAT <u>TG</u> ACG          | <u>ag</u> ttaatgga | 138 |
|    | monocytogenes  |                     |                       |                     |        |                    |                              |                    |     |
|    | Micrococcus    | GGAAGTCCGT          | GAGTTGCTGG            | CTGCCCAGGA          | ATTCAA | <u>Gregicer</u> g  | TCIGICACAC                   | <b>agttga</b> tgga | 159 |
| 10 | luteus         |                     |                       |                     |        |                    |                              |                    |     |
|    | Neisseria      | GGAAATCCGC          | GGAAATCCGC GACCTGCTGT | CCAGCTACGA          | CTT    | A <u>cg</u> aagaa  | AAAATCTTCG                   | AACTGGCTAC         | 162 |
|    | gonorrhoeae    |                     |                       |                     |        |                    |                              |                    |     |
|    | Salmonella     | GGAAGTTCGC          | GGAAGTTCGC GAACTGCTGT | CTCAGTACGA          | CTT    | GGGAAGCG           | GGGAAGCG AAAATCATCG          | AACTGGCTGG         | 164 |
|    | typhimurium    |                     |                       | į.                  |        |                    |                              |                    |     |
| 15 | Staphylococcus | GGAAGTTCGT          | GACTTALTAB            | GCGAATATGA CTT.     | CTT    | · · · CGAAGAA      | AAAATCTTAG                   | AATTAATGGA         | 140 |
|    | aureus         |                     |                       |                     |        |                    |                              |                    |     |
|    | Streptococcus  | GGAAATCCGT          | GACCTAITGT            | CAGAATACGA          | CTT    | · · · CGAAGAC      | AICGLIATGG                   | <b>B</b> ATTGATGAA | 145 |
|    | pneumoniae     |                     |                       |                     |        |                    |                              |                    |     |
|    | Treponema      | agaggt <u>g</u> cgt | <u>Gate</u> cectte    | CTGGATATGG          | GTTGGA | GGATGCAGCT         | TG <u>TA</u> T <u>TG</u> AGG | AACTGCTTGC         | 169 |
| 20 | 20 pallidum    |                     |                       |                     |        |                    |                              |                    |     |
|    |                |                     |                       |                     |        |                    |                              |                    |     |

| • | Selected         | CAAGAAG GTTGGTTACA ACCCAAAGA | ATCCGGTAAA GTTACTGGTA AGACCT |
|---|------------------|------------------------------|------------------------------|
|   | sednences        |                              |                              |
|   | Selected         | SEQ ID NO: 11                | SEQ ID NO: 12ª               |
| 2 | species-specific |                              |                              |
|   | primer           | CAAGAAG GTTGGTTACA ACCCAAAGA | AGGICTIACC AGTAACTITAC CGGAT |
|   | sequences:       |                              |                              |

Underlined nucleotides gene fragment. 10 The sequence numbering refers to the Candida albicans tuf match that identical to the selected sequence

This sequence is the reverse-complement of the above tuf sequence.

.. GGCAC GTATGATGAG CCAGGCGATG CGTAAGCTGG

.. AGCTC GACTAATGTC TCAAGCACTA

TTAGAGATIG CCGAIGCCII AGIITCAAGT GGTGC.

Enterococcus

faecium

20

Escherichia

coli

CTGGAAAICT GTGACGCCCI GGCGCGTTCT GGCGC.

| Annex V:              | Strategy for the        | for the selection from the recA        | from the re      | gene of      | the amplification             | ion primers specific for   | អ   |
|-----------------------|-------------------------|--|------------------|--------------|-------------------------------|--|-----|
|                       | the genus <i>Strept</i> | Streptococcus (co                      | (continues on    | pages 62 and | 63).                          |  |     |
|                       | 415                     |  |                  | 449540       |                               | 574 S  | SEQ |
|                       |                         |  |                  |              |                               | I QI   | NO  |
| Bordetella            | CTCGAGATCA              | Toboboveou                             | genecates        | GGCTCGGCC    | . GGCCC GCCTGATGAG CCAGGCGCTG | CCAGGCGCTG CGCAAGCTGA  |     |
| pertussis             |                         |  |                  |              |                               |  |     |
| Burkholderia          | CTCGAAAICA CCGAIGCGCI   |  | GGIGCGCTCG GGCTC | GGCTCGGCCC   | CCCIGAIGIC GCAGGCGCIG         | GCAGGCGCIG CGCAAGCTGA  |     |
| cepacia               |                         |  |                  |              |                               |  |     |
| ${\it Campylobacter}$ | TTAGAAATTG              | TTAGAAATTG TAGAAACTAT                  | AGCAAGAAGT GGCGC |              | AGCAA GACITAIGIC              | TCAAGCTCIA AGAAAACTTA  |     |
| ) jejuni              |                         |  |                  |              |                               |  |     |
| Chlamydia             | TTGAGTATTG              | CAGAGCTCTT                             | AGCGCGTTCT GGAGC |              | C GCATGAIGTC                  | .AGCTC GC <u>AIGAIG</u> TC G <u>CAGGC</u> TC <u>IA CGCAA</u> ATTAA |     |
| trachomatis           |                         |  |                  |              |                               |  |     |
| ${\it Clostridium}$   | TTAGAAATAA              | TTAGAAATAA CAGAAGCTIT                  | AGIIAGATCA GGAGC |              | A GATIAAIGTC                  | AGCTA GATIAAIGTC ACAAGCCTIA AGAAAGTTAA                             |     |
| perfringens           |                         |  |                  |              |                               |  |     |
| s Corynebacterium     | _                       | CTGGAGATIG CAGAIATGCI TGIICGCTCT GGAGC | TGIICGCICT       |              | C GTTTGATGAG                  | AGCGC GTTTGATGAG TCAGGCGCTG CGTAAGATGA                             |     |
| pseudotuberculosis    | osis                    |  |                  |              |                               |  |     |
| Enterobacter          | CTGGAAAICT              | CTGGAAAICT GTGAIGCGCI GACCCGTTCA GGCGC | GACCCGTTCA       |              | C GTATGATGAG                  | AGCTC GTATGATGAG CCAGGCGATG CGTAAGCTTG                             |     |
| adalomerans           |                         |  |                  |              |                               |  |     |

|    | Haemophilus     | gcgaacagaa gaatagaatt ttaatgcatt accgcgacct gtgagtttac gcaaagcttg agacattaaa  |
|----|-----------------|---|
|    | influenzae      |   |
|    | Helicobacter    | TTA <u>gaaatt</u> t tagaaacgat caccagaagc ggaggagcaa ggc <u>ttatgag ccatgcgtta agaaa</u> aatca                                  |
|    | pylori          |   |
| u) | 5 Lactococcus   | CTTCAAATIG CIGAAAAII GAIIACTTCI GGAGCAGCAC GIAIGAIGIC ACAAGCCAIG CGIAAACTIG   |
|    | lactis          |   |
|    | Legionella      | CTG <u>GAAATTA CTGATA</u> TGC <u>T GGT</u> GCGTTCT GCAGCGGCAA GAT <u>TGATG</u> TC G <u>CAAGCCCTG CGTAA</u> ATTGA                |
|    | pneumophila     |   |
|    | Mycoplasma      | TTTGCTC <u>TT</u> A TCGAATCATT AATTAAACA AACAATGCAA GA <u>ATGATG</u> TC AAAAGGTTTG CGAAGAATAC                                   |
| 10 | ) genitalium    |   |
|    | Neisseria       | TTG <u>GARAT</u> CT GCGACACGCI CGICCGTTCG GGCGGGGCGC GCC <u>TGATGAG ICAGGC</u> TTIG CGCAAACTGA                                  |
|    | gonorrhoeae     |   |
|    | Proteus         | CTGGAAATIT GTGAIGCAIT ATCICGCTCT GGTGCCGCAC GTAIGAIGAG CCAAGCTAIG CGIAAACTAG  |
|    | mirabilis       |   |
| 15 | 5 Pseudomonas   | CTG <u>GAAAT</u> CA <u>CCGACA</u> TGC <u>T</u> <u>GGT</u> GCGCTCC AACGCGGCAC GCC <u>TGATG</u> TC C <u>CAGGCGCTG CGCAA</u> GATCA |
|    | aeruginosa      |   |
|    | Serratia        | CTGGAAAICT GTGAIGCGCI GACCCGCTCC GGCGCGGCGC GCATGAIGAG CCAGGCGAIG CGIAAGCTGG  |
|    | marcescens      |   |
|    | Shigella        | CTGGAAAICT GTGACGCCCI GGCGCGTTCT GGCGCGGCAC GTAIGAIGAG CCAGGCGAIG CGIAAGCTGG  |
| 20 | ) flexneri      |   |
|    | Staphylococcus  | CTT <u>GAAATCG CCGAAGCATT TGTTAGAAGT</u> GGTGCAGCTC GTT <u>TAATG</u> TC A <u>CAAGCGTTA CGTAA</u> ACTTT                          |
|    | aureus          |   |
|    | Streptococcus   | TTAGAAATIG CAGGAAAATI GAIIGACTCI GGGGC  |
|    | gordonii        |   |
| 25 | 5 Streptococcus | CTTGAAATTG CAGGGAAATT GATTGATTCT GGCGCAGCAC GCATGATGAG TCAAGCGATG CGTAAATTAT 33   |
|    | mutans          |   |

|    |                       |                                  |  |       |         |                              |                            |                    |      | sednences":    |    |
|----|-----------------------|----------------------------------|--|-------|---------|------------------------------|----------------------------|--------------------|------|----------------|----|
|    | ATCAT                 | TTACGCAT GGCITGACTC ATCAT        | TTACGCAT   |       |         | GATTGA                       | GGIAAATT                   | GAAATTG CAGGIAAATT |      | primer         |    |
|    |                       |                                  |  |       |         |                              |                            |                    | n    | genus-specific | 15 |
|    | 22 <sup>b</sup>       | SEQ ID NO: 2                     | V.   |       |         | 21                           | SEQ ID NO: 2               | SEC                |      | Selected       |    |
|    |                       |                                  |  |       |         |                              |                            |                    |      |                |    |
|    |                       |                                  |  |       |         |                              |                            |                    |      | sednences      |    |
|    | CGTAA                 | TCAIGCCATG CGTAA                 | ATGATGAG   |       |         | GATTGA                       | CAGGIAAATT GATTGA          | GAAATIG CO         |      | Selected       |    |
|    |                       |                                  |  |       |         |                              |                            |                    |      | pestis         | 10 |
|    | CCAGGCTATG CGTAAGCTGG | CCAGGCTATG                       | cecec grateateag   | :     | r gerec | GIGAIGCGCI GACICGCTCT GGTGC. | r <u>gat</u> gcgc <u>t</u> | CTGGAAATTT GI      | CTC  | Yersinia       |    |
|    |                       |                                  |  |       |         |                              | •                          |                    |      | cholerae       |    |
| _  | CGTAAACTGA            | GCAAGCAATG                       | AGCGC GIATGTTGTC GCAAGCAATG CGTAAACTGA                                       |       | T GGTGC | GGCICGCTCT GGTGC.            | GTGAIGCACI                 | CTGGAAATTT GI      | CTC  | Vibrio         |    |
|    |                       |                                  |  |       |         |                              |                            |                    |      | salivarius     |    |
| 36 | CGTAAACTTT            | GTATGATGAG TCAAGCCATG CGTAAACTTT |  | AGCGC | T GGTGC | GATTGACTCT GGTGC.            | CAGGIAAGCI                 | CTCGAAATTG CA      | CTC  | Streptococcus  | īU |
|    |                       |                                  |  |       |         |                              |                            |                    |      | pyogenes       |    |
| 35 | CGTAAATTAT            | TCAGGCCATG                       | AGCAC GTATGATGAG TCAGGCCATG  | •     | T GGTGC | CAGGTAAATT GATTGATTCT GGTGC. | GGTAAATT                   | CTTGAAATTG CA      | CTJ  | Streptococcus  |    |
|    |                       |                                  |  |       |         |                              |                            |                    |      | pneumoniae     |    |
| 4  | CGTAAAC171G           | CCAGGCCATG                       | CTTGAGATIG CGGGAAATI GATIGACICA GGIGCGGCIC GIAIGAIGAG CCAGGCCAIG CGIAAACITIG | GGCTC | A GGTGC | <u>GATTGACTC</u>             | GGAAAATT                   | CCAGATTG CC        | E CE | Streptococcus  |    |

recA sequence. Underlined nucleotides S. pneumoniae sednence or match that The sequence numbering refers to the seguence selected to the 20

identical

are

any of the four nucleotides can bind that a nucleotide analog "I" stands for inosine which

sequence. the above recA This sequence is the reverse complement of

Annex VI: Specific and ubiquitous primers for DNA amplification

| SEQ I            | NO Nucleotide sequence                | Originating | DNA fragment |
|------------------|---------------------------------------|-------------|--------------|
|                  |                                       | SEQ ID      | Nucleotide   |
|                  |                                       | NO          | position     |
| Bacte:           | cial species: Enterococcus faecium    |             |              |
|                  |                                       |             |              |
| 1                | 5'-TGC TTT AGC AAC AGC CTA TCA G      | 26ª         | 273-294      |
| 2 <sub>p</sub>   | 5'-TAA ACT TCT TCC GGC ACT TCG        | 26ª         | 468-488      |
| Bacte            | rial species: Listeria monocytogenes  |             |              |
| 3                | 5'-TGC GGC TAT AAA TGA AGA GGC        | 27ª         | 339-359      |
| 4 <sup>b</sup>   | 5'-ATC CGA TGA TGC TAT GGC TTT        | 27ª         | 448-468      |
| Bacte            | rial species: Neisseria meningitidis  |             |              |
| 5                | 5'-CCA GCG GTA TTG TTT GGT GGT        | 28°         | 56-76        |
| . 6 <sub>p</sub> | 5'-CAG GCG GCC TTT AAT AAT TTC        | 28ª         | 212-232      |
| Bacter           | ial species: Staphylococcus saprophyt | icus        |              |
| 7                | 5'- AGA TCG AAT TCC ACA TGA AGG TTA T | TA TGA 29°  | 290-319      |
| 8 <sub>p</sub>   | 5'- TCG CTT CTC CCT CAA CAA TCA AAC T | 'AT CCT 29° | 409-436      |
| <u>Bacter</u>    | ial species: Streptococcus agalactiae |             |              |
| 9                | 5'-TTT CAC CAG CTG TAT TAG AAG TA     | 30ª         | 59-81        |
| 10 <sup>b</sup>  | 5'-GTT CCC TGA ACA TTA TCT TTG AT     | 30ª         | 190-212      |
| Fungal           | species: Candida albicans             |             |              |
| 11               | 5'-CAA GAA GGT TGG TTA CAA CCC AAA GA | . 120°      | 61-86        |
| 12 <sup>b</sup>  | 5'-AGG TCT TAC CAG TAA CTT TAC CGG AT | 120°        | 184-209      |

<sup>\*</sup> Sequences from databases.

<sup>35</sup> b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

<sup>&</sup>lt;sup>c</sup> Sequences determined by our group.

Annex VI: Specific and ubiquitous primers for DNA amplification (continues on next page)

|    | SEQ ID          | NO N     | ucle      | otid        | e se  | quen  | ce      |     |     |   | Originating            | DNA fragment         |
|----|-----------------|----------|-----------|-------------|-------|-------|---------|-----|-----|---|------------------------|----------------------|
|    |                 |          |           |             |       |       |         |     |     |   | SEQ ID                 | Nucleotide           |
|    |                 |          |           |             |       |       |         |     |     |   | NO                     | position             |
| 5  | Bacter:         | ial genu | <u>s:</u> | Ente        | rocc  | occus | 3       |     |     |   |                        |                      |
|    | 13              | 5'-TAC   | TGA       | CAA         | ACC   | ATT   | CAT     | GAT | G   |   | 131-134 <sup>a,b</sup> | 319-340°             |
|    | 14 <sup>d</sup> | 5'-AAC   | TTC       | GTC         | ACC   | AAC   | GCG     | AAC |     |   | 131-134 <sup>a,b</sup> | 410-430°             |
| 10 | Bacter          | ial genu | s:        | Neis        | seri  | ia    |         |     |     |   |                        |                      |
| 10 | 15              | 5'-CTG   | GCG       | CGG         | ייעיי | CCT   | CGG     | тт  |     |   | 31°                    | 21-40 <sup>t</sup>   |
|    | 16ª             | 5'-GCC   |           |             |       |       |         |     | G   |   | 31°                    | 102-123 <sup>t</sup> |
|    |                 | ial genu |           | Stap        |       |       |         |     | Ū   |   | 32                     | 101 113              |
|    |                 | ·        |           | _           | ٠.    |       |         |     |     |   |                        |                      |
| 15 | 17              | 5'-CCG   | TGT       | TGA         | ACG   | TGG   | TCA     | AAT | CAA | A | 140-143a,b             | 391-415 <sup>9</sup> |
|    | 18ª             | 5'-TRT   | GTG       | GTG         | TRA   | TWG   | WRC     | CAG | GAG | C | 140-143 <sup>a,b</sup> | 584-608 <sup>9</sup> |
|    | 19              | 5'-ACA   | ACG       | TGG         | WCA   | AGT   | WTT     | AGC | WGC | T | 140-143 <sup>a,b</sup> | 562-583 <sup>g</sup> |
|    | 20 <sup>d</sup> | 5'-ACC   | ATT       | TCW         | GTA   | CCT   | TCT     | GGT | AAG | T | 140-143 <sup>a,b</sup> | 729-753 <sup>9</sup> |
| 20 | Bacter          | ial genu | s:        | Stre        | ptoc  | occu  | ıs      |     | •   |   | •                      |                      |
|    | w               |          |           | ;<br>;      |       |       |         |     |     |   |                        |                      |
|    | . 21            | 5'-GAA   | ATT       | GCA         | GGI   | AAA   | TTG     | ATT | GA  |   | 32-36°                 | 418-440 <sup>h</sup> |
|    | 22ª             | 5'-TTA   | CGC       | ATG         | GCI   | TGA   | CTC     | ATC | AT  |   | 32-36°                 | 547-569h             |
| 25 |                 |          |           | Univ        | /ers  | al pi | rimer   | :8  |     |   |                        |                      |
|    | 23              | 5'-ACI   | KKI       | ACI         | GGI   | GTI   | GAR     | ARG | TT  |   | 118-146 <sup>a,b</sup> | 493-515 <sup>i</sup> |
|    |                 |          |           |             |       |       |         |     |     |   | 147-171ª.e             |                      |
|    | 24 <sup>d</sup> | 5'-AYR   | TTI       | TCI         | CCI   | GGC   | ATI     | ACC | AT  |   | 118-146 <sup>a,b</sup> | 778-800°             |
|    |                 |          | <u></u>   | <del></del> |       |       | <u></u> |     |     |   | 147-171 <sup>a,e</sup> | <del></del>          |

- 30 \* These sequences were aligned to derive the corresponding primer.
  - b tuf sequences determined by our group.
  - $^{\rm c}$  The nucleotide positions refer to the  $\it E.$  faecalis tuf gene fragment (SEQ ID NO: 132).
- These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.
  - \* Sequences from databases.
  - t The nucleotide positions refer to the N. meningitidis asd gene fragment (SEQ ID NO: 31).

- $^{9}$  The nucleotide positions refer to the S. aureus tuf gene fragment (SEQ ID NO: 140).
- <sup>h</sup> The nucleotide positions refer to the S. pneumoniae recA gene (SEQ ID NO: 34).
- 5 i The nucleotide positions refer to the E. coli tuf gene fragment (SEQ ID NO: 154).

Annex VI: Specific and ubiquitous primers for DNA amplification

|    | SEQ ID          | NO Nucleotide sequence               | Originating | DNA fragment      |
|----|-----------------|--------------------------------------|-------------|-------------------|
|    |                 |                                      | SEQ ID      | Nucleotide        |
|    |                 |                                      | NO          | position          |
| _  | Antibio         | otic resistance gene: blate          |             |                   |
| 5  | 37              | 5'-CTA TGT GGC GCG GTA TTA TC        | -           | -                 |
|    | 38              | 5'-CGC AGT GTT ATC ACT CAT GG        | -           | -                 |
|    | 39              | 5'-CTG AAT GAA GCC ATA CCA AA        | -           | -                 |
| 10 | 40              | 5'-ATC AGC AAT AAA CCA GCC AG        | -           | -                 |
|    | Antibio         | otic resistance gene: blashy         |             |                   |
|    | 41              | 5'-TTA CCA TGA GCG ATA ACA GC        |             |                   |
|    | 42              | 5'-CTC ATT CAG TTC CGT TTC CC        | _           | <del>-</del><br>- |
| 15 | **              | 5 CIC MII CAG IIC CGI IIC CC         | _           | -                 |
|    | 43              | 5'-CAG CTG CTG CAG TGG ATG GT        | -           | -                 |
|    | 44              | 5'-CGC TCT GCT TTG TTA TTC GG        | <b>-</b>    | -                 |
|    | Antibio         | otic resistance gene: blarob         |             |                   |
| 20 |                 |                                      |             | :                 |
|    | 45              | 5'-TAC GCC AAC ATC GTG GAA AG        | -           | -                 |
| :  | 46              | 5'-TTG AAT TTG GCT TCT TCG GT        | -           | -                 |
|    | 47              | 5'-GGG ATA CAG AAA CGG GAC AT        | <b>-</b> .  | <b>-</b>          |
| 25 | 48              | 5'-TAA ATC TTT TTC AGG CAG CG        | -           | - '               |
|    | Antibio         | otic resistance gene: bla oxa        |             |                   |
|    | 49              | 5'-GAT GGT TTG AAG GGT TTA TTA TAA G | 110ª        | 686-710           |
| 30 | 50 <sup>b</sup> | 5'-AAT TTA GTG TGT TTA GAA TGG TGA T | 110ª        | 802-826           |
|    | Antibio         | otic resistance gene: blaz           |             |                   |
|    | 51              | 5'-ACT TCA ACA CCT GCT GCT TTC       | 111ª        | 511-531           |
| 35 | 52 <sup>b</sup> | 5'-TGA CCA CTT TTA TCA GCA ACC       | 111ª        | 663-683           |
|    | Antibio         | otic resistance gene: aadB           |             | •                 |
|    | 53              | 5'-GGC AAT AGT TGA AAT GCT CG        | -           | -                 |
| 40 | 54              | 5'-CAG CTG TTA CAA CGG ACT GG        | -           | -                 |
|    | Antibio         | otic resistance gene: aacC1          |             |                   |
|    | 55              | 5'-TCT ATG ATC TCG CAG TCT CC        | -           | <del>-</del> .    |
| 45 | 56              | 5'-ATC GTC ACC GTA ATC TGC TT        |             | -                 |
|    |                 |                                      |             | <del></del>       |

Sequences from databases.

## SUBSTITUTE SHEET (RULE 26)

b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI: Specific and ubiquitous primers for DNA amplification

| SEQ             | ID NO Nu          | ucleotide        | s seđr   | ience      | _    | Originating | DNA fragmen     |
|-----------------|-------------------|------------------|----------|------------|------|-------------|-----------------|
|                 |                   |                  |          |            |      | SEQ ID      | Nucleotide      |
|                 |                   |                  |          |            |      | NO          | position        |
| Anti            | biotic res        | istance (        | gene:    | aacC2      | ?    |             | ·               |
| 57              | 5!_ሮ <u>ል</u> ሞ ' | TCT CGA          | ው የነው    | יייי יייכר | ጥል   | _           |                 |
| 58              |                   | AAA TGC          |          |            |      | -           | -<br>-          |
| Anti            | biotic resi       | istance (        | gene ·   | aacC3      | 1    |             |                 |
| <u> </u>        | <u> </u>          |                  | <u> </u> |            | •    |             |                 |
| 59              | 5'-CTG (          | GAT TAT          | GGC T    | AC GGA     | GT   | -           | -               |
| 60              | 5'-AGC            | AGT GTG          | ATG G    | TA TCC     | AG   | -           | -               |
| Anti            | biotic resi       | istance (        | gene:    | aac6'      | -IIa |             |                 |
| 61              | קי-מא <i>ר</i> י  | TCT TGA          | ת מב     | ርጥ ርርጥ     | GG   | 112ª        | 123-14          |
| 62 <sup>b</sup> |                   | GTC TAT          |          |            |      | 112ª        | 284-30          |
| <b>V</b> 2      |                   |                  | 100 1    |            |      | 4.4.4       | 204 - 50        |
| 63              | 5'-TAT (          | GAG AAG          | GCA G    | GA TTC     | GT   | 112ª        | 445-46          |
| 64 <sup>b</sup> | 5'-GCT '          | TTC TCT          | CGA A    | GG CTT     | GT · | 112ª        | 522-54          |
| Anti            | biotic res        | <u>istance (</u> | gene:    | aacA4      |      |             |                 |
| 65              | 5'-GAG '          | TTG CTG          | TTC A    | AT GAT     | CC . | _           | -               |
| 66              | 5'-GTG'           | TTT GAA          | CCA T    | GT ACA     | CG " | -           | -               |
| Anti            | biotic resi       | istance (        | gene:    | aad(6      | 54)  |             |                 |
| 173             | 5'-TCT '          | TTA GCA          | GAA C    | AG GAT     | GAA  | _           | , <u>-</u>      |
| 174             | 5'-GAA '          | TAA TTC          | ATA T    | CC TCC     | G    | -           | -               |
| <u>Anti</u>     | biotic resi       | istance (        | gene:    | vanA       |      |             |                 |
| 67              | 5'-TGT 2          | AGA GGT          | CTA G    | CC CGT     | GT   | -           | -               |
| 68              | 5'-ACG            | GGG ATA          | ACG A    | CT GTA     | TG   | -           | -               |
| 69              | 5'-ATA            | AAG ATG .        | ATA G    | GC CGG     | TG   | _           | ••              |
| 70              |                   | TGT CAT          |          |            | •    | <b>-</b> .  | -               |
| Anti            | biotic res        | istance (        | gene:    | vanB       |      |             |                 |
| 71              | ፍ t _ አምጥ         | ATC TTC          | פפר פ    | ביי שוביר  | ፐር   | 116ª        | 22-41           |
| 72 <sup>b</sup> |                   | TAT CGG          |          |            |      | 116ª        | 171- <b>1</b> 9 |
|                 |                   |                  |          |            |      |             |                 |
| 73              | 5'-CGA            | TAG AAG          | CAG C    | AG GAC     | AA   | 116ª        | 575-59          |

<sup>&</sup>lt;sup>a</sup> Sequences from databases.

## SUBSTITUTE SHEET (RULE 26)

b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI: Specific and ubiquitous primers for DNA amplification

| SEQ I           | D NO Nucleotide sequence               | Originating | DNA fragment |
|-----------------|--|-------------|--------------|
|                 |  | SEQ ID      | Nucleotide   |
|                 |  | NO          | position     |
| Antib           | piotic resistance gene: vanC           |             |              |
| 75              | 5'-GCC TTA TGT ATG AAC AAA TGG         | 117ª        | 373-39       |
| 76 <sup>b</sup> | 5'-GTG ACT TTW GTG ATC CCT TTT GA      | 117ª        | 541-56       |
| Antib           | oiotic resistance gene: msrA           |             |              |
| 77              | 5'-TCC AAT CAT TGC ACA AAA TC          | -           | -            |
| 78              | 5'-AAT TCC CTC TAT TTG GTG GT          | -           | -            |
| 79              | 5'-TCC CAA GCC AGT AAA GCT AA          | -           | -            |
| 80              | 5'-TGG TTT TTC AAC TTC TTC CA          | -           | -            |
| Antib           | iotic resistance gene: satA            |             |              |
| 81              | 5'-TCA TAG AAT GGA TGG CTC AA          | -           | -            |
| 82              | 5'-AGC TAC TAT TGC ACC ATC CC          | -           | -            |
| Antib           | iotic resistance gene: aac(6')-aph(2") | •           |              |
| 83              | 5'-CAA TAA GGG CAT ACC AAA AAT C       | -           | · -          |
| 84              | 5'-CCT TAA CAT TTG TGG CAT TAT C       | -           | -            |
| 85              | 5'-TTG GGA AGA TGA AGT TTT TAG A       | -           | -            |
| 86              | 5'-CCT TTA CTC CAA TAA TTT GGC T       | -           | -            |
| Antib           | iotic resistance gene: vat             |             |              |
| 87              | 5'-TTT CAT CTA TTC AGG ATG GG          | ••          | -            |
| 88              | 5'-GGA GCA ACA TTC TTT GTG AC          | ~           | -            |
| Antib           | oiotic resistance gene: vga            |             |              |
| 89              | 5'-TGT GCC TGA AGA AGG TAT TG          | -           | -            |
| 90              | 5'-CGT GTT ACT TCA CCA CCA CT          | -           | -            |
| Antib           | piotic resistance gene: ermA           |             |              |
| 91              | 5'-TAT CTT ATC GTT GAG AAG GGA TT      | 113ª        | 370-39       |
| 92 <sup>b</sup> | 5'-CTA CAC TTG GCT TAG GAT GAA A       | 113ª        | 487-50       |

<sup>45</sup> a Sequences from databases.

b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI: Specific and ubiquitous primers for DNA amplification

| SEQ I                        | D NO Nucleotide sequence               | Origin | ating DNA  |
|------------------------------|--|--------|------------|
|                              |  | fragme | nt         |
|                              |  | SEQ    | Nucleotide |
|                              |  | ID NO  | position   |
| Antib                        | iotic resistance gene: ermB            |        |            |
| 93                           | 5'-CTA TCT GAT TGT TGA AGA AGG ATT     | 114ª   | 366-389    |
| 94 <sup>b</sup>              | 5'-GTT TAC TCT TGG TTT AGG ATG AAA     | 114ª   | 484-50     |
| Antib                        | iotic resistance gene: ermC            |        |            |
| 95                           | 5'-CTT GTT GAT CAC GAT AAT TTC C       | 115*   | 214-23!    |
| <sup>.</sup> 96 <sup>b</sup> | 5'-ATC TTT TAG CAA ACC CGT ATT C       | 115ª   | 382-403    |
| Antib                        | iotic resistance gene: mecA            |        |            |
| 97                           | 5'-AAC AGG TGA ATT ATT AGC ACT TGT AAG | -      | -          |
| 98                           | 5'-ATT GCT GTT AAT ATT TTT TGA GTT GAA | -      | -          |
| Antib                        | iotic resistance gene: int             |        |            |
| 99                           | 5'-GTG ATC GAA ATC CAG ATC C           | -      | -          |
| 100                          | 5'-ATC CTC GGT TTT CTG GAA G           | -      |            |
| 101                          | 5'-CTG GTC ATA CAT GTG ATG G           | -      | •          |
| 102                          | 5'-GAT GTT ACC CGA GAG CTT G           | -      | -          |
| Antib                        | iotic resistance gene: sul             |        |            |
| 103                          | 5'-TTA AGC GTG CAT AAT AAG CC          | -      | -          |
| 104                          | 5'-TTG CGA TTA CTT CGC CAA CT          | -      | -          |
| 105                          | 5'-TTT ACT AAG CTT GCC CCT TC          | -      | -          |
| 106                          | 5'-AAA AGG CAG CAA TTA TGA GC          | _      | _          |

<sup>35</sup> Sequences from databases.

b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

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#### SEQUENCE LISTING

#### (1) GENERAL INFORMATION:

- (i) APPLICANT:
  - (A) NAME: INFECTIO DIAGNOSTIC (I.D.I.) INC.
  - (B) STREET: 2050, BOULEVARD RENE LEVESQUE OUEST, 4E ETAGE
  - (C) CITY: STE-FOY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1V 2K8
  - (G) TELEPHONE: (418) 681-4343
  - (H) TELEFAX: (418) 681-5254
  - (A) NAME: BERGERON, MICHEL G.
  - (B) STREET: 2069 RUE BRULARD
  - (C) CITY: SILLERY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1T 1G2
  - (A) NAME: PICARD, FRANCOIS J.
  - (B) STREET: 1245, RUE DE LA SAPINIERE
  - (C) CITY: CAP-ROUGE
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1Y 1A1
  - (A) NAME: OUELLETTE, MARC
  - (B) STREET: 1035 DE PLOERMEL
  - (C) CITY: SILLERY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1S 3S1
  - (A) NAME: ROY, PAUL H.
  - (B) STREET: 28, RUE CHARLES GARNIER
  - (C) CITY: LORETTEVILLE
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G2A 3S1
- (ii) TITLE OF INVENTION: SPECIES-SPECIFIC, GENIUS-SPECIFIC AND UNIVERSAL DNA PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL AND FUNGAL PATHOGENS AND ASSOCIATED ANTIBIOTIC RESISTANCE GENES ...
- (iii) NUMBER OF SEQUENCES: 174
- (iv) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) PRIOR APPLICATION DATA:

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| (A) APPLICATION N<br>(B) FILING DATE:  | NUMBER: US 08/743,637<br>04-NOV-1996 |    |
|--|--------------------------------------|----|
| (2) INFORMATION FOR SEQ ID   | NO: 1:                               |    |
| (i) SEQUENCE CHARACTER  (A) LENGTH: 22 ba  (B) TYPE: nucleio  (C) STRANDEDNESS:  (D) TOPOLOGY: lin | ase pairs<br>c acid<br>c single      |    |
| (ii) MOLECULE TYPE: DNA  | A (genomic)                          |    |
| (vi) ORIGINAL SOURCE: (A) ORGANISM: Ent  | cerococcus faecium                   |    |
| (xi) SEQUENCE DESCRIPTI  | ON: SEQ ID NO: 1:                    |    |
| TGCTTTAGCA ACAGCCTATC AG   |                                      | 22 |
| (2) INFORMATION FOR SEQ ID   | NO: 2:                               |    |
| (i) SEQUENCE CHARACTER  (A) LENGTH: 21 ba  (B) TYPE: nucleio  (C) STRANDEDNESS:  (D) TOPOLOGY: lin | se pairs<br>acid<br>single           |    |
| (ii) MOLECULE TYPE: DNA  | (genomic)                            |    |
| (vi) ORIGINAL SOURCE: (A) ORGANISM: Ent  | cerococcus faecium                   |    |
| (xi) SEQUENCE DESCRIPTI  | ON: SEQ ID NO: 2:                    |    |
| TAAACTTCTT CCGGCACTTC G  | 2                                    | 21 |
| (2) INFORMATION FOR SEQ ID   | NO: 3:                               |    |
| (i) SEQUENCE CHARACTER  (A) LENGTH: 21 ba  (B) TYPE: nucleic  (C) STRANDEDNESS:  (D) TOPOLOGY: lin | se pairs<br>cacid<br>single          |    |
| (ii) MOLECULE TYPE: DNA  | (genomic)                            |    |
| (vi) ORIGINAL SOURCE:<br>(A) ORGANISM: Lis   | steria monocytogenes                 |    |
| (xi) SEQUENCE DESCRIPTI  | ON: SEQ ID NO: 3:                    |    |
| TGCGGCTATA AATGAAGAGG C  | 2                                    | 21 |
| (2) INFORMATION FOR SEQ ID   | NO: 4:                               |    |

|     |       | (A) LENGTH: 21 base pairs            |       |
|-----|-------|--------------------------------------|-------|
|     |       | (B) TYPE: nucleic acid               |       |
|     |       | (C) STRANDEDNESS: single             |       |
|     |       | (D) TOPOLOGY: linear                 |       |
|     | (ii)  | MOLECULE TYPE: DNA (genomic)         |       |
|     | (vi)  | ORIGINAL SOURCE:                     |       |
|     |       | (A) ORGANISM: Listeria monocytogenes |       |
|     | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 4:  |       |
| ATC | CGATG | AT GCTATGGCTT T                      | 21    |
| (2) | INFO  | RMATION FOR SEQ ID NO: 5:            |       |
|     | (i)   | SEQUENCE CHARACTERISTICS:            |       |
|     |       | (A) LENGTH: 21 base pairs            |       |
|     |       | (B) TYPE: nucleic acid               |       |
|     |       | (C) STRANDEDNESS: single             |       |
|     |       | (D) TOPOLOGY: linear                 |       |
|     | (ii)  | MOLECULE TYPE: DNA (genomic)         |       |
|     | (vi)  | ORIGINAL SOURCE:                     | ,     |
|     |       | (A) ORGANISM: Neisseria meningitidis |       |
|     | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 5:  | 1.1 A |
| CCA | GCGGT | AT TGTTTGGTGG T                      | 21    |
| (2) | INFO  | RMATION FOR SEQ ID NO: 6:            |       |
|     |       |                                      |       |
|     | (i)   | SEQUENCE CHARACTERISTICS:            |       |
|     |       | (A) LENGTH: 21 base pairs            |       |
|     |       | (B) TYPE: nucleic acid               |       |
|     |       | (C) STRANDEDNESS: single             |       |
|     |       | (D) TOPOLOGY: linear                 |       |
|     | (ii)  | MOLECULE TYPE: DNA (genomic)         |       |
|     | (vi)  | ORIGINAL SOURCE:                     |       |
|     |       | (A) ORGANISM: Neisseria meningitidis |       |
|     | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 6:  |       |
| CAG | GCGGC | CT TTAATAATTT C                      | 21    |
| (2) | INFO  | RMATION FOR SEQ ID NO: 7:            |       |
|     | (i)   | SEQUENCE CHARACTERISTICS:            |       |
|     |       | (A) LENGTH: 30 base pairs            |       |
|     |       | (B) TYPE: nucleic acid               |       |
|     |       | (C) STRANDEDNESS: single             |       |
|     |       | (D) TOPOLOGY: linear                 |       |

|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|------|--------|--|----|
|      | (vi)   | ORIGINAL SOURCE: (A) ORGANISM: Staphylococcus saprophyticus  |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 7:  |    |
| AGA' | rcgaa' | TT CCACATGAAG GTTATTATGA   | 30 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 8:  |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 30 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (vi)   | ORIGINAL SOURCE: (A) ORGANISM: Staphylococcus saprophyticus  |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 8:  |    |
| TCG  | CTTCT  | CC CTCAACAATC AAACTATCCT   | 30 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 9:  |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 23 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (vi)   | ORIGINAL SOURCE: (A) ORGANISM: Streptococcus agalactiae  |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 9:  |    |
| TTTC | CACCA  | GC TGTATTAGAA GTA  | 23 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 10:   |    |
| ٠    | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 23 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (vi)   | ORIGINAL SOURCE:  (A) ORGANISM: Streptococcus agalactiae   |    |

|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 10:   |    |
|------|--------|--|----|
| GTT  | CCCTG  | AA CATTATCTTT GAT  | 23 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 11:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (vi)   | ORIGINAL SOURCE: (A) ORGANISM: Candida albicans  |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 11:   |    |
| CAAC | GAAGG' | TT GGTTACAACC CAAAGA   | 26 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 12:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear | ,  |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (vi)   | ORIGINAL SOURCE: (A) ORGANISM: Candida albicans  |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 12:   |    |
| AGG" | CTTA   | CC AGTAACTTTA CCGGAT   | 26 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 13:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 13:   |    |
| TAC  | rgaca: | AA CCATTCATGA TG   | 22 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 14:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs  |    |

|      |        | (B) TYPE: nucleic acid   |    |
|------|--------|--|----|
|      |        | (C) STRANDEDNESS: single   |    |
|      |        | (D) TOPOLOGY: linear   |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   | •  |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 14:   |    |
| AAC' | TTCGT  | CA CCAACGCGAA C  | 21 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 15:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 15:   |    |
| CTG  | GCGCG  | GT ATGGTCGGTT  | 20 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 16:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 16:   |    |
| GCC  | GACGT' | TG GAAGTGGTAA AG   | 22 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 17:   |    |
|      |        | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 25 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 17:   |    |
| CCGT | rgttgi | AA CGTGGTCAAA TCAAA  | 25 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 18:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 base pairs  |    |

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| <ul><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>   |    |
|--|----|
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:  |    |
| TRTGTGGTGT RATWGWRCCA GGAGC  | 25 |
| (2) INFORMATION FOR SEQ ID NO: 19:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 25 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:  |    |
| ACAACGTGGW CAAGTWTTAG CWGCT  | 25 |
| (2) INFORMATION FOR SEQ ID NO: 20:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 25 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:  |    |
| ACCATTTCWG TACCTTCTGG TAAGT  | 25 |
| (2) INFORMATION FOR SEQ ID NO: 21:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 23 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:12     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:  |    |
| GAAATTGCAG GNAAATTGAT TGA  | 23 |

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## (2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

#### TTACGCATGG CNTGACTCAT CAT

23

# (2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:6
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 9
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:15
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

| ACNKKNACNG GNGTNGARAT GTT  | 23 |
|--|----|
| (2) INFORMATION FOR SEQ ID NO: 24:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 23 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:6     (D) OTHER INFORMATION:/note= "n = inosine"</pre>  |    |
| <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:9     (D) OTHER INFORMATION:/note= "n = inosine"</pre>  |    |
| <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:12     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
| <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:18     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:  |    |
| AYRTTNTCNC CNGGCATNAC CAT  | 23 |
| (2) INFORMATION FOR SEQ ID NO: 25:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 10 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  | •  |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:  |    |
| TCGCTTCTCC   | 10 |
| (2) INFORMATION FOR SEQ ID NO: 26:   |    |
| <ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 600 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: double</li></ul>                                   |    |

- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Enterococcus faecium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTCTTAGAGA CATTGAATAT GCCTTATGTC GGCGCAGGCG TATTGACCAG TGCATGTGCC 60 ATGGATAAAA TCATGACCAA GTATATTTTA CAAGCTGCTG GTGTGCCGCA AGTTCCTTAT 120 GTACCAGTAC TTAAGAATCA ATGGAAAGAA AATCCTAAAA AAGTATTTGA TCAATGTGAA 180 GGTTCTTTGC TTTATCCGAT GTTTGTCAAA CCTGCGAATA TGGGTTCTAG TGTCGGCATT 240 ACAAAGGCAG AAAACCGAGA AGAGCTGCAA AATGCTTTAG CAACAGCCTA TCAGTATGAT 300 TCTCGAGCAA TCGTTGAACA AGGAATTGAA GCGCGCGAAA TCGAAGTTGC TGTATTAGGA 360 AATGAAGATG TTCGGACGAC TTTGCCTGGC GAAGTCGTAA AAGACGTAGC ATTCTATGAT 420 TATGAAGCCA AATATATCAA TAATAAAATC GAAATGCAGA TTCCAGCCGA AGTGCCGGAA 480 GAAGTTTATC AAAAAGCGCA AGAGTACGCG AAGTTAGCTT ACACGATGTT AGGTGGAAGC 540 GGATTGAGCC GGTGCGATTT CTTTTTGACA AATAAAAATG AATTATTCCT GAATGAATTA 600

# (2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1920 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Listeria monocytogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTGGGATTAA ACAGATTTAT GCGTGCGATG ATGGTGGTTT TCATTACTGC CAATTGCATT 60

ACGATTAACC CCGACATAAT ATTTGCAGCG ACAGATAGCG AAGATTCTAG TCTAAACACA 120

GATGAATGGG AAGAAGAAAA AACAGAAGAG CAACCAAGCG AGGTAAATAC GGGACCAAGA 180

TACGAAACTG CACGTGAAGT AAGTTCACGT GATATTAAAG AACTAGAAAA ATCGAATAAA 240

GTGAGAAATA CGAACAAAGC AGACCTAATA GCAATGTTGA AAGAAAAAGC AGAAAAAGGT 300

CCCAAATATCA ATAATAACAA CAGTGAACAA ACTGAGAATG CGGCTATAAA TGAAGAGGCT 360

| TCAGGAGCCG  | ACCGACCAGC  | TATACAAGTG  | GAGCGTCGTC | ATCCAGGATT | GCCATCGGAT | 420  |
|-------------|-------------|-------------|------------|------------|------------|------|
| AGCGCAGCGG  | AAATTAAAA   | AAGAAGGAAA  | GCCATAGCAT | CATCGGATAG | TGAGCTTGAA | 480  |
| AGCCTTACTT  | ATCCGGATAA  | ACCAACAAAA  | GTAAATAAGA | AAAAAGTGGC | GAAAGAGTCA | 540  |
| GTTGCGGATG  | CTTCTGAAAG  | TGACTTAGAT  | TCTAGCATGC | AGTCAGCAGA | TGAGTCTTCA | 600  |
| CCACAACCTT  | TAAAAGCAAA  | CCAACAACCA  | TTTTTCCCTA | AAGTATTTAA | ААААТАААА  | 660  |
| GATGCGGGGA  | AATGGGTACG  | TGATAAAATC  | GACGAAAATC | CTGAAGTAAA | GAAAGCGATT | 720  |
| GTTGATAAAA  | GTGCAGGGTT  | AATTGACCAA  | TTATTAACCA | AAAAGAAAAG | TGAAGAGGTA | 780  |
| AATGCTTCGG  | ACTTCCCGCC  | ACCACCTACG  | GATGAAGAGT | TAAGACTTGC | TTTGCCAGAG | 840  |
| ACACCAATGC  | TTCTTGGTTT  | TAATGCTCCT  | GCTACATCAG | AACCGAGCTC | ATTCGAATTT | 900  |
| CCACCACCAC  | CTACGGATGA  | AGAGTTAAGA  | CTTGCTTTGC | CAGAGACGCC | AATGCTTCTT | 960  |
| GGTTTTAATG  | CTCCTGCTAC  | ATCGGAACCG  | AGCTCGTTCG | AATTTCCACC | GCCTCCAACA | 1020 |
| GAAGATGAAC  | TAGAAATCAT  | CCGGGAAACA  | GCATCCTCGC | TAGATTCTAG | TTTTACAAGA | 1080 |
| GGGGATTTAG  | CTAGTTTGAG  | AAATGCTATT  | AATCGCCATA | GTCAAAATTT | CTCTGATTTC | 1140 |
| CCACCAATCC  | CAACAGAAGA  | AGAGTTGAAC  | GGGAGAGGCG | GTAGACCAAC | ATCTGAAGAA | 1200 |
| TTTAGTTCGC  | TGAATAGTGG  | TGATTTTACA  | GATGACGAAA | ACAGCGAGAC | AACAGAAGAA | 1260 |
| GAAATTGATC  | GCCTAGCTGA  | TTTAAGAGAT  | AGAGGAACAG | GAAAACACTC | AAGAAATGCG | 1320 |
| GGTTTTTTAC  | CATTAAATCC  | GTTTGCTAGC  | AGCCCGGTTC | CTTCGTTAAG | TCCAAAGGTA | 1380 |
| TCGAAAATAA  | GCGACCGGGC  | TCTGATAAGT  | GACATAACTA | AAAAAACGCC | ATTTAAGAAT | 1440 |
| CCATCACAGC  | CATTAAATGT  | GTTTAATAAA  | AAAACTACAA | CGAAAACAGT | GACTAAAAAA | 1500 |
| CCAACCCCTG  | TAAAGACCGC  | ACCAAAGCTA  | GCAGAACTTC | CTGCCACAAA | ACCACAAGAA | 1560 |
| ACCGTACTTA  | GGGAAAATAA  | AACACCCTTT  | ATAGAAAAAC | AAGCAGAAAC | AAACAAGCAG | 1620 |
| TCAATTAATA  | TGCCGAGCCT  | ACCAGTAATC  | CAAAAAGAAG | CTACAGAGAG | CGATAAAGAG | 1680 |
| GAAATGAAAC  | CACAAACCGA  | GGAAAAAATG  | GTAGAGGAAA | GCGAATCAGC | TAATAACGCA | 1740 |
| AACGGAAAAA  | ATCGTTCTGC  | TGGCATTGAA  | GAAGGAAAAC | TAATTGCTAA | AAGTGCAGAA | 1800 |
| GACGAAAAAG  | CGAAGGAAGA  | ACCAGGGAAC  | CATACGACGT | TAATTCTTGC | AATGTTAGCT | 1860 |
| ATTGGCGTGT  | TCTCTTTAGG  | GGCGTTTATC  | AAAATTATTC | AATTAAGAAA | AATTAATTAA | 1920 |
| (2) INFORMA | TION FOR SE | O ID NO: 28 | ) <u>.</u> |            |            |      |

(2) INFORMATION FOR SEQ ID NO: 28:

<sup>(</sup>i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 base pairs

200 200

| <ul><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: double</li><li>(D) TOPOLOGY: linear</li></ul>  |     |
|---|-----|
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE:  (A) ORGANISM: Neisseria meningitidis   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:   |     |
| TACCGGTACG CTAAATATTG GTGATGTATT GGATATTATG ATTTGGGAAG CGCCGCCAGC   | 60  |
| GGTATTGTTT GGTGGTGGCC TTTCTTCGAT GGGCTCGGGT AGTGCGCAAC AAACCAAGTT   | 120 |
| GCCGGAGCAA CTGGTGACGG CACGTGGTAC GGTTTCTGTG CCGTTTGTTG GCGATATTTC   | 180 |
| GGTGGTCGGT AAAACGCCTG GTCAGGTTCA GGAAATTATT AAAGGCCGCC TGAAAAAAT  | 240 |
| GGCCAATCAG CCGCAAGTGA TGGTGCGCTT GGTGCAGAAT AATGCGGCAA ATGTATCGGT   | 300 |
| GATTCGCGCA GGCAATAGTG TGCGTATGCC GTTGACGGCA GCCGGTGAGC GTGTGTTGGA   | 360 |
| TGCGGTGGCT GCGGTAGGTG GTTCAACGGC AAATGTGCAG GATACGAATG TGCAG  | 415 |
| <br>(2) INFORMATION FOR SEQ ID NO: 29:  |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 438 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> | 1,  |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE:  (A) ORGANISM: Staphylococcus saprophyticus   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:   |     |
| TCGCTTCTCC AGAAGAAATT TTAGAAACAT ATCTAGAAAA TCCCAAATTA GATAAACCGT   | 60  |
| TTATATTATG TGAATACGCA CATGCAATGG GAAATTCACC AGGAGATCTT AATGCATATC   | 120 |
| AAACATTAAT TGAAAAATAT GATAGTTTTA TTGGCGGTTT TGTTTGGGAA TGGTGTGATC   | 180 |
| ATAGCATTCA GGTTGGGATA AAGGAAGGTA AACCAATTTT TAGATATGGT GGAGATTTTG   | 240 |
| GTGAGGCCTT ACATGACGGT AATTTTTGTG TTGATGGTAT TGTTTCGCCA GATCGAATTC   | 300 |
| CACATGAAGG TTATTATGAG TTTAAACATG AACATAGACC TTTGAGATTG GTTAACGAAG   |     |
| AGGATTATCG GTTTACATTG AAGAATCAAT TTGATTTTAC AAATGCGGAG GATAGTTTGA   | 360 |
| TTGTTGAGGG AGAAGCGA   | 420 |
| IIIIIIUUU MUMMULUM  | 420 |

| (2) INFORMATION FOR SEQ ID NO: 3 | (2) | INFORMATION | FOR | SEO | ID | NO: | 30 |
|----------------------------------|-----|-------------|-----|-----|----|-----|----|
|----------------------------------|-----|-------------|-----|-----|----|-----|----|

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 768 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus agalactiae
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ATGAACGTTA CACATATGAT GTATCTATCT GGAACTCTAG TGGCTGGTGC ATTGTTATTT 60 TCACCAGCTG TATTAGAAGT ACATGCTGAT CAAGTGACAA CTCCACAAGT GGTAAATCAT 120 GTAAATAGTA ATAATCAAGC CCAGCAAATG GCTCAAAAGC TTGATCAAGA TAGCATTCAG 180 TTGAGAAATA TCAAAGATAA TGTTCAGGGA ACAGATTATG AAAAACCGGT TAATGAGGCT 240 ATTACTAGCG TGGAAAAATT AAAGACTTCA TTGCGTGCCA ACCCTGAGAC AGTTTATGAT 300 TTGAATTCTA TTGGTAGTCG TGTAGAAGCC TTAACAGATG TGATTGAAGC AATCACTTTT 360 TCAACTCAAC ATTTAACAAA TAAGGTTAGT CAAGCAAATA TTGATATGGG ATTTGGGATA 420 ACTAAGCTAG TTATTCGCAT TTTAGATCCA TTTGCTTCAG TTGATTCAAT TAAAGCTCAA 480 GTTAACGATG TAAAGGCATT AGAACAAAAA GTTTTAACTT ATCCTGATTT AAAACCAACT 540 GATAGAGCTA CCATCTATAC AAAATCAAAA CTTGATAAGG AAATCTGGAA TACACGCTTT 600 ACTAGAGATA AAAAAGTACT TAACGTCAAA GAATTTAAAG TTTACAATAC TTTAAATAAA 660 GCAATCACAC ATGCTGTTGG AGTTCAGTTG AATCCAAATG TTACGGTACA ACAAGTTGAT 720 CAAGAGATTG TAACATTACA AGCAGCACTT CAAACAGCAT TAAAATAA 768

- (2) INFORMATION FOR SEO ID NO: 31:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 421 base pairs
    - (B) TYPE: nucleic acid(C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Neisseria meningitidis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

| ATGAAAGTAG GTTTCGTCGG CTGGCGCGGT ATGGTCGGTT CGGTTTTGAT GCAGCGTATG   | 60  |
|---|-----|
| AAAGAAGAAA ACGACTTCGC CCACATTCCC GAAGCGTTTT TCTTTACCAC TTCCAACGTC   | 120 |
| GGCGGCGCAC GCCCTGATTT CGGTCAGGCG GCTAAAACAT TATTGGACGC GAACAACGTT   | 180 |
| GCCGAGCTGG CAAAAATGGA CATCATCGTT ACCTGCCAAG GCGGCGACTA CACCAAATCC   | 240 |
| GTCTTCCAAG CCCTGCGCGA CAGCGGCTGG AACGGCTACT GGATTGACGC GGCATCCTCG   | 300 |
| CTGCGTATGA AAGACGACGC GATTATCGTC CTCGACCCCG TCAACCGCAA CGTCATCGAC   | 360 |
| AACGGCCTCA AAAACGGCGT GAAAAACTAC ATCGGCGGCA ACTGTACCGT TTCCCTGATG   | 420 |
| <b>c</b>  | 421 |
| (2) INFORMATION FOR SEQ ID NO: 32:  |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS: <ul> <li>(A) LENGTH: 213 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> </li> <li>(ii) MOLECULE TYPE: DNA (genomic)</li> <li>(vi) ORIGINAL SOURCE: <ul> <li>(A) ORGANISM: Streptococcus gordonii</li> </ul> </li> </ul> |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:   |     |
| TTCATAGACG CTGAGCACGC TTTGGATCCA TCTTACGCGG CTGCTCTAGG TGTAAATATT   | 60  |
| GATGAGCTGT TGCTATCTCA ACCAGATTCT GGTGAGCAAG GTTTAGAAAT TGCAGGAAAA   | 120 |
| TTGATTGACT CTGGGGCAGT TGATTTAGTT GTCATCGACT CTGTTGCAGC TCTTGTACCA   | 180 |
| CGTGCGGAAA TCGATGGAGA TATCGGTGAT AGC  | 213 |
| (2) INFORMATION FOR SEQ ID NO: 33:  |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 692 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul>   |     |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE:  (A) ORGANISM: Streptococcus mutans   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:   |     |
| GGCCGGAAT CTTCTGGTAA GACAACTGTC GCTCTTCATG CTGCTGCTCA GGCGCAAAA   | 60  |

| GATGGCGGTA | TTGCCGCTTT | CATTGATGCA | GAACATGCCC | TTGATCCAGC | CTATGCTGCT | 120 |
|------------|------------|------------|------------|------------|------------|-----|
| GCTCTTGGCG | TTAATATTGA | TGAGCTTTTG | CTTTCACAAC | CAGATTCAGG | AGAACAGGGT | 180 |
| CTTGAAATTG | CAGGGAAATT | GATTGATTCT | GGCGCTGTTG | ATTTAGTTGT | TGTTGACTCA | 240 |
| GTGGCAGCTT | TAGTACCACG | TGCGGAGATT | GACGGAGATA | TTGGTAATAG | TCATGTTGGC | 300 |
| TTACAAGCAC | GCATGATGAG | TCAAGCGATG | CGTAAATTAT | CAGCTTCAAT | CAATAAAACA | 360 |
| AAAACCATTG | CTATTTTTAT | TAATCAATTG | CGGGAAAAAG | TTGGTATTAT | GTTTGGTAAT | 420 |
| CCAGAAACAA | CCCCTGGCGG | GCGTGCCTTG | AAGTTTTATT | CTTCTGTGCG | TCTTGATGTC | 480 |
| CGCGGCAATA | CTCAAATTAA | AGGAACCGGG | GAACAAAAAG | ACAGCAATAT | TGGTAAAGAG | 540 |
| ACCAAAATTA | AAGTTGTTAA | AAATAAAGTT | GCTCCACCAT | TTAAGGAAGC | TTTTGTAGAA | 600 |
| ATTATATATG | GTGAAGGCAT | TTCTCGTACA | GGTGAATTAG | TTAAGATTGC | CAGTGATTTG | 660 |
| GGAATTATCC | AAAAAGCTGG | AGCTTGGTAC | TC         |            |            | 692 |

### (2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1204 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus pneumoniae
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ATGGCGAAAA AACCAAAAAA ATTAGAAGAA ATTTCAAAAA AATTTGGGGC AGAACGTGAA 60 AAGGCCTTGA ATGACGCTCT TAAATTGATT GAGAAAGACT TTGGTAAAGG ATCAATCATG 120 CGTTTGGGTG AACGTGCGGA GCAAAAGGTG CAAGTGATGA GCTCAGGTTC TTTAGCTCTT 180 GACATTGCCC TTGGCTCAGG TGGTTATCCT AAGGGACGTA TCATCGAAAT CTATGGCCCA 240 GAGTCATCTG GTAAGACAAC GGTTGCCCTT CATGCAGTTG CACAAGCGCA AAAAGAAGGT 300 GGGATTGCTG CCTTTATCGA TGCGGAACAT GCCCTTGATC CAGCTTATGC TGCGGCCCTT 360 GGTGTCAATA TTGACGAATT GCTCTTGTCT CAACCAGACT CAGGAGAGCA AGGTCTTGAG 420 ATTGCGGGAA AATTGATTGA CTCAGGTGCA GTTGATCTTG TCGTAGTCGA CTCAGTTGCT 480 GCCCTTGTTC CTCGTGCGGA AATTGATGGA GATATCGGAG ATAGCCATGT TGGTTTGCAG 540 GCTCGTATGA TGAGCCAGGC CATGCGTAAA CTTGGCGCCT CTATCAATAA AACCAAAACA 600

| ATTGCCATTT | TTATCAACCA | ATTGCGTGAA | AAAGTTGGAG | TGATGTTTGG | AAATCCAGAA | 660  |
|------------|------------|------------|------------|------------|------------|------|
| ACAACACCGG | GCGGACGTGC | TTTGAAATTC | TATGCTTCAG | TCCGCTTGGA | TGTTCGTGGT | 720  |
| AATACACAAA | TTAAGGGAAC | TGGTGATCAA | AAAGAAACCA | ATGTCGGTAA | AGAAACTAAG | 780  |
| ATTAAGGTTG | ТАААААТАА  | GGTAGCTCCA | CCGTTTAAGG | AAGCCGTAGT | TGAAATTATG | 840  |
| TACGGAGAAG | GAATTTCTAA | GACTGGTGAG | CTTTTGAAGA | TTGCAAGCGA | TTTGGATATT | 900  |
| ATCAAAAAAG | CAGGGGCTTG | GTATTCTTAC | AAAGATGAAA | AAATTGGGCA | AGGTTCTGAG | 960  |
| AATGCTAAGA | AATACTTGGC | AGAGCACCCA | GAAATCTTTG | ATGAAATTGA | TAAGCAAGTC | 1020 |
| CGTTCTAAAT | TTGGCTTGAT | TGATGGAGAA | GAAGTTTCAG | AACAAGATAC | TGAAAACAAA | 1080 |
| AAAGATGAGC | CAAAGAAAGA | AGAAGCAGTG | AATGAAGAAG | TTCCGCTTGA | CTTAGGCGAT | 1140 |
| GAACTTGAAA | TCGAAATTGA | AGAATAAGCT | GTTAAAGCAG | TGGAGAAATC | CGCTACTTTT | 1200 |
| TCGA       |            |            |            |            |            | 1204 |

## (2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 981 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear:
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus pyogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ATGCGTTCAG GAAGTCTAGC TCTTGATATT GCTTGGATAG CTGGTGGTTA TCCTAAAGGA CGTATCATCG AAATCTATGG TCCAGAGTCT TCCGGTAAAA CGACTGTGGC TTTACATGCT 120 GTAGCACAAG CTCAAAAAGA AGGTGGAATC GCAGCCTTTA TCGATGCCGA GCATGCGCTT 180 GATCCAGCTT ATGCTGCTGC GCTTGGGGTT AATATTGATG AACTTCTCTT GTCTCAACCA 240 GATTCTGGAG AACAAGGACT TGAAATTGCA GGTAAATTGA TTGATTCTGG TGCGGTTGAC 300 CTGGTTGTTG TCGATTCAGT AGCAGCTTTA GTGCCACGTG CTGAAATTGA TGGTGATATT 360 GGCGATAGCC ATGTCGGATT GCAAGCACGT ATGATGAGTC AGGCCATGCG TAAATTATCA 420 GCTTCTATTA ATAAAACAAA AACTATCGCA ATCTTTATCA ACCAATTGCG TGAAAAAGTT 480 GGTGTGATGT TTGGAAATCC TGAAACAACA CCAGGTGGTC GAGCTTTGAA ATTCTATGCT 540 TCTGTTCGGC TGGATGTGCG TGGAAACAAC CAAATTAAAG GAACTGGTGA CCAAAAGATA 600

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| GCCAGCATTG GTAAGGAGAC CAAAATCAAG GTTGTTAAAA ACAAGGTCGC TCCGCCATTT   | 66  |
|---|-----|
| AAGGTAGCAG AAGTTGAAAT CATGTATGGG GAAGGTATTT CTCGTACAGG GGAGCTTGTG   | 72  |
| AAAATTGCTT CTGATTTGGA CATTATCCAA AAAGCAGGTG CTTGGTTCTC TTATAATGGT   | 78  |
| GAGAAGATTG GCCAAGGTTC TGAAAATGCT AAGCGTTATT TGGCCGATCA TCCACAATTG   | 84  |
| TTTGATGAAA TCGACCGTAA AGTACGTGTT AAATTTGGTT TGCTTGAAGA AAGCGAAGAA   | 90  |
| GAATCTGCTA TGGCAGTAGC ATCAGAAGAA ACCGATGATC TTGCTTTAGA TTTAGATAAT   | 960 |
| GGTATTGAAA TTGAAGATTA A   | 98: |
| (2) INFORMATION FOR SEQ ID NO: 36:  |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 312 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> |     |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE: (A) ORGANISM: Streptococcus salivarius  |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:   |     |
| GCGTATGCAC GAGCTCTAGG TGTTAATATC GATGAGCTTC TTTTGTCGCA GCCTGATTCT   | 60  |
| GGTGAGCAAG GTCTCGAAAT TGCAGGTAAG CTGATTGACT CTGGTGCAGT GGATTTAGTT   | 120 |
| GTTGTTGACT CAGTTGCGGC CTTCGTACCA CGTGCAGAAA TTGATGGAGA TAGTGGTGAC   | 180 |
| AGTCATGTAG GACTTCAAGC GCGTATGATG AGTCAAGCCA TGCGTAAACT TTCTGCATCT   | 24( |
| ATTAATAAAA CAAAAACGAT TGCTATCTTT ATTAACCAGT TGCGTGAAAA AGTTGGTATC   | 300 |
| ATGTTTGGTA AC   | 312 |
| (2) INFORMATION FOR SEQ ID NO: 37:  |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>  |     |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:   |     |
| CTATGTGGCG CGGTATTATC   | 20  |
| (2) INFORMATION FOR SEQ ID NO: 38:  |     |

| <ul> <li>(i) SEQUENCE CHARACTERISTICS:         <ul> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> </li> </ul> |    |
|--|----|
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:  |    |
| CGCAGTGTTA TCACTCATGG  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 39:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>                     |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:  |    |
| CTGAATGAAG CCATACCAAA  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 40:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>                     |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:  |    |
| ATCAGCAATA AACCAGCCAG  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 41:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>                     |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:  |    |
| TTACCATGAG CGATAACAGC  | 20 |
| (2) INFORMATION FOR SEC ID NO. 42.   |    |

|      | (i)  | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|------|------|--|----|
|      | (ii) | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi) | SEQUENCE DESCRIPTION: SEQ ID NO: 42:   |    |
| CTCA | TTCA | GT TCCGTTTCCC  | 20 |
| (2)  | INFO | RMATION FOR SEQ ID NO: 43:   |    |
|      | (i)  | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii) | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi) | SEQUENCE DESCRIPTION: SEQ ID NO: 43:   |    |
| CAGO | TGCT | GC AGTGGATGGT  | 20 |
| (2)  | INFO | RMATION FOR SEQ ID NO: 44:   |    |
|      | (i)  | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii) | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi) | SEQUENCE DESCRIPTION: SEQ ID NO: 44:   |    |
| CGCT | CTGC | TT TGTTATTCGG  | 20 |
| (2)  | INFO | RMATION FOR SEQ ID NO: 45:   |    |
|      | (i)  | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii) | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi) | SEQUENCE DESCRIPTION: SEQ ID NO: 45:   |    |
| TACG | CCAA | CA TCGTGGAAAG  | 20 |
| (2)  | INFO | RMATION FOR SEQ ID NO: 46:   |    |

| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|--|----|
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:  |    |
| TTGAATTTGG CTTCTTCGGT  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 47:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:  |    |
| GGGATACAGA AACGGGACAT  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 48:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:  |    |
| TAAATCTTTT TCAGGCAGCG  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 49:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 25 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:  |    |
| GATGGTTTGA AGGGTTTATT ATAAG  | 25 |
| (2) INFORMATION FOR SEQ ID NO: 50:   |    |

|      | (i)   | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 25 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|------|-------|--|----|
|      | (ii)  | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 50:   |    |
| AATT | TAGT  | ST GTTTAGAATG GTGAT  | 25 |
| (2)  | INFO  | RMATION FOR SEQ ID NO: 51:   |    |
|      | (i)   | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)  | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 51:   |    |
| ACTT | CAAC  | AC CTGCTGCTTT C  | 21 |
| (2)  | INFO  | RMATION FOR SEQ ID NO: 52:   |    |
|      | (i)   | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear | •. |
|      | (ii)  | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 52:   |    |
| TGAC | CACTI | TT TATCAGCAAC C  | 21 |
| (2)  | INFOR | RMATION FOR SEQ ID NO: 53:   |    |
|      | (i)   | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)  | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)  | SEQUENCE DESCRIPTION: SEQ ID NO: 53:   |    |
| GGCA | ATAGI | TT GAAATGCTCG  | 20 |
| (2)  | INFOR | RMATION FOR SEQ ID NO: 54:   |    |

|      | <ul><li>(A) LENGTH: 20 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>   |    |
|------|--|----|
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:  |    |
| CAG  | CTGTTAC AACGGACTGG   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 55:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:  |    |
| TCT  | ATGATCT CGCAGTCTCC   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 56:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:  |    |
| ATC  | STCACCG TAATCTGCTT   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 57:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:  |    |
| CAT' | TCTCGAT TGCTTTGCTA   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 58:   |    |

|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|------|--------|--|----|
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 58:   |    |
| CCG  | AAATG  | CT TCTCAAGATA  | 20 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 59:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 59:   |    |
| CTG  | SATTA' | rg gctacggagt  | 20 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 60:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 60:   |    |
| AGC  | AGTGT  | GA TGGTATCCAG  | 20 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 61:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 61:   |    |
| GAC' | rcttg: | AT GAAGTGCTGG  | 20 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 62:   |    |

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|     | <ul> <li>(i) SEQUENCE CHARACTERISTICS:         <ul> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> </li> </ul> |    |
|-----|--|----|
|     | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|     | xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:   |    |
| CTG | CTATT CCTCGCACTC   | 20 |
| (2) | NFORMATION FOR SEQ ID NO: 63:  |    |
|     | <ul> <li>(i) SEQUENCE CHARACTERISTICS:         <ul> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> </li> </ul> |    |
|     | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|     | xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:   |    |
| TAT | GAAGG CAGGATTCGT   | 20 |
| (2) | NFORMATION FOR SEQ ID NO: 64:  |    |
|     | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>                     |    |
|     | ii) MOLECULE TYPE: DNA (genomic)   |    |
|     | xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:   |    |
| GCT | CTCTC GAAGGCTTGT   | 20 |
| (2) | NFORMATION FOR SEQ ID NO: 65:  |    |
|     | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>                     |    |
|     | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|     | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:  |    |
| GAG | CGCTGT TCAATGATCC  | 20 |
| (2) | INFORMATION FOR SEQ ID NO: 66:   |    |

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|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |      |     |
|------|--------|--|------|-----|
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |      |     |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 66:   |      |     |
| GTG' | TTTGA  | AC CATGTACACG  | 20   |     |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 67:   |      |     |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |      |     |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |      |     |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 67:   |      |     |
| TGT  | AGAGG  | TC TAGCCCGTGT  | 20 . | ·   |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 68:   | •    | - , |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear | . :  | 7   |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |      |     |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 68:   |      |     |
| ACG  | GGGAT. | AA CGACTGTATG  | 20   |     |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 69:   |      |     |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |      |     |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |      |     |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 69:   |      |     |
| ATA  | AAGAT  | TGA TAGGCCGGTG   | 20   |     |

(2) INFORMATION FOR SEQ ID NO: 70:

|      | <ul><li>(A) LENGTH: 20 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>   |    |
|------|--|----|
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:  |    |
| TGC  | IGTCATA TTGTCTTGCC .   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 71:   |    |
|      | <ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 20 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>       |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:  |    |
| ATT  | ATCTTCG GCGGTTGCTC .   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 72:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:  |    |
| GACI | TATCGGC TTCCCATTCC   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 73:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:  |    |
| CGAT | TAGAAGC AGCAGGACAA   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 74:   |    |

|      | (i)             | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|------|-----------------|--|----|
|      | ( <b>i</b> i)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)            | SEQUENCE DESCRIPTION: SEQ ID NO: 74:   |    |
| CTG  | ATGGA'          | TG CGGAAGATAC  | 20 |
| (2)  | INFO            | RMATION FOR SEQ ID NO: 75:   |    |
|      | (i)             | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)            | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)            | SEQUENCE DESCRIPTION: SEQ ID NO: 75:   |    |
| GCC: | rta <b>t</b> g' | TA TGAACAAATG G .  | 21 |
| (2)  | INFO            | RMATION FOR SEQ ID NO: 76:   | ,  |
|      | (i)             | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 23 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii):           | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)            | SEQUENCE DESCRIPTION: SEQ ID NO: 76:   |    |
| GTG! | ACTTT           | WG TGATCCCTTT TGA  | 23 |
| (2)  | INFO            | RMATION FOR SEQ ID NO: 77:   |    |
|      | (i)             | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)            | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)            | SEQUENCE DESCRIPTION: SEQ ID NO: 77:   |    |
| TCC  | AATCA           | TT GCACAAATC   | 20 |
| (2)  | INFO            | RMATION FOR SEQ ID NO: 78:   |    |

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|      | (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid   |    |
|------|--|----|
|      | (C) STRANDEDNESS: single   |    |
|      | (D) TOPOLOGY: linear   |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:  |    |
| AAT" | TCCCTCT ATTTGGTGGT   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 79:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:  |    |
| TCC  | CAAGCCA GTAAAGCTAA   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 80:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:  |    |
| TGG' | TTTTTCA ACTTCTTCCA   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 81:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:  |    |
| TCA  | TAGAATG GATGGCTCAA   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 82:   |    |

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| (i)           | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|---------------|--|----|
| (ii)          | MOLECULE TYPE: DNA (genomic)   |    |
| (xi)          | SEQUENCE DESCRIPTION: SEQ ID NO: 82:   |    |
| AGCTACTA      | TT GCACCATCCC  | 20 |
| (2) INFO      | RMATION FOR SEQ ID NO: 83:   |    |
| (i)           | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
| (ii)          | MOLECULE TYPE: DNA (genomic)   |    |
| (xi)          | SEQUENCE DESCRIPTION: SEQ ID NO: 83:   |    |
| CAATAAGG      | GC ATACCAAAAA TC   | 22 |
| (2) INFO      | RMATION FOR SEQ ID NO: 84:   |    |
| (i)           | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
| (i <b>i</b> ) | MOLECULE TYPE: DNA (genomic)   |    |
| (xi)          | SEQUENCE DESCRIPTION: SEQ ID NO: 84:   |    |
| CCTTAACA      | TT TGTGGCATTA TC   | 22 |
| (2) INFO      | RMATION FOR SEQ ID NO: 85:   |    |
| <b>(i)</b>    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
| (ii)          | MOLECULE TYPE: DNA (genomic)   |    |
| (xi)          | SEQUENCE DESCRIPTION: SEQ ID NO: 85:   |    |
| TTGGGAAG      | SAT GAAGTTTTTA GA  | 22 |

(2) INFORMATION FOR SEQ ID NO: 86:

| <ul><li>(A) LENGTH: 22 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>   |    |
|--|----|
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:  |    |
| CCTTTACTCC AATAATTTGG CT   | 22 |
| (2) INFORMATION FOR SEQ ID NO: 87:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:  |    |
| TTTCATCTAT TCAGGATGGG  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 88:   |    |
| (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear   |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:  |    |
| GGAGCAACAT TCTTTGTGAC  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 89:   |    |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
| (ii) MOLECULE TYPE: DNA (genomic)  |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:  |    |
| TGTGCCTGAA GAAGGTATTG  | 20 |
| (2) INFORMATION FOR SEQ ID NO: 90:   |    |

|      | (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear   |    |
|------|--|----|
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:  |    |
| CGT  | GTTACTT CACCACCACT   | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 91:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 23 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:  |    |
| TAT  | CTTATCG TTGAGAAGGG ATT   | 23 |
| (2)  | INFORMATION FOR SEQ ID NO: 92:   | :. |
| •    | (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear   |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:  |    |
| CTA  | CACTTGG CTTAGGATGA AA  | 22 |
| (2)  | INFORMATION FOR SEQ ID NO: 93:   |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 24 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:  |    |
| CTA' | TCTGATT GTTGAAGAAG GATT  | 24 |
| (2)  | TNFOPMATTON FOR SEC ID NO. 94.   |    |

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|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 24 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|------|--------|--|----|
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 94:   |    |
| GTTI | TACTC' | TT GGTTTAGGAT GAAA   | 24 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 95:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 95:   |    |
| CTT  | STTGA' | TC ACGATAATTT CC   | 22 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 96:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 96:   |    |
| ATC  | CTTTA  | GC AAACCCGTAT TC   | 22 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 97:   |    |
|      | (i)    | SEQUENCE CHARACTERISTICS:  (A) LENGTH: 27 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear |    |
|      | (ii)   | MOLECULE TYPE: DNA (genomic)   |    |
|      | (xi)   | SEQUENCE DESCRIPTION: SEQ ID NO: 97:   |    |
| AAC  | AGGTG  | AA TTATTAGCAC TTGTAAG  | 27 |
| (2)  | INFO   | RMATION FOR SEQ ID NO: 98:   |    |

| (1) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 27 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear   |        |
|--|--------|
| (ii) MOLECULE TYPE: DNA (genomic)  |        |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:  |        |
| ATTGCTGTTA ATATTTTTTG AGTTGAA  | 27     |
| (2) INFORMATION FOR SEQ ID NO: 99:   |        |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 19 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |        |
| (ii) MOLECULE TYPE: DNA (genomic)  |        |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:  | ,      |
| GTGATCGAAA TCCAGATCC   | 19     |
| (2) INFORMATION FOR SEQ ID NO: 100:  |        |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 19 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> | · ½ .3 |
| (ii) MOLECULE TYPE: DNA (genomic)  |        |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:   |        |
| ATCCTCGGTT TTCTGGAAG   | 19     |
| (2) INFORMATION FOR SEQ ID NO: 101:  (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 19 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear                |        |
| (ii) MOLECULE TYPE: DNA (genomic)  |        |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:   |        |
| CTGGTCATAC ATGTGATGG   | 19     |

(2) INFORMATION FOR SEQ ID NO: 102:

|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 19 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|------|--|----|
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:   |    |
| GATG | TTACCC GAGAGCTTG   | 19 |
| (2)  | INFORMATION FOR SEQ ID NO: 103:  |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  | ٠  |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:   |    |
| TTAA | GCGTGC ATAATAAGCC  | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 104:  |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:   |    |
| TTGC | GATTAC TTCGCCAACT  | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 105:  |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:   |    |
| TTTA | CTAAGC TTGCCCCTTC  | 20 |
| (2)  | INFORMATION FOR SEQ ID NO: 106:  |    |

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(i) SEQUENCE CHARACTERISTICS:

|     | <ul><li>(A) LENGTH: 20 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: single</li><li>(D) TOPOLOGY: linear</li></ul>   |    |
|-----|--|----|
|     | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|     | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:   |    |
| AAA | AGGCAGC AATTATGAGC   | 20 |
| (2) | INFORMATION FOR SEQ ID NO: 107:  |    |
|     | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 29 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|     | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|     | <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:9     (D) OTHER INFORMATION:/note= "n = inosine"</pre>  |    |
|     | <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:12     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
|     | <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:15     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
|     | <pre>(ix) FEATURE:     (A) NAME/KEY: misc_feature     (B) LOCATION:18     (D) OTHER INFORMATION:/note= "n = inosine"</pre>   |    |
|     | <pre>(ix) FEATURE:      (A) NAME/KEY: misc_feature      (B) LOCATION:21      (D) OTHER INFORMATION:/note= "n = inosine"</pre>  |    |
|     | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:   |    |
| AAY | ATGATNA CNGGNGCNGC NCARATGGA   | 29 |
| (2) | INFORMATION FOR SEQ ID NO: 108:  |    |
|     | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs  |    |

(B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 6
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 9
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

# CCNACNGTNC KNCCRCCYTC RCG

23

- (2) INFORMATION FOR SEQ ID NO: 109:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 29 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
    - (ii) MOLECULE TYPE: DNA (genomic)
    - (ix) FEATURE:
      - (A) NAME/KEY: misc\_feature
        - (B) LOCATION:6
      - (D) OTHER INFORMATION:/note= "n = inosine"
    - (ix) FEATURE:
      - (A) NAME/KEY: misc\_feature
      - (B) LOCATION:12
        - (D) OTHER INFORMATION:/note= "n = inosine"
    - (ix) FEATURE:
      - (A) NAME/KEY: misc\_feature
      - (B) LOCATION:15
      - (D) OTHER INFORMATION:/note= "n = inosine"
    - (ix) FEATURE:
      - (A) NAME/KEY: misc\_feature

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- (B) LOCATION:18
- (D) OTHER INFORMATION: /note= "n = inosine"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

#### CARYTNATHG TNGCNGTNAA YAARATGGA

29

- (2) INFORMATION FOR SEQ ID NO: 110:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 831 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

| ATGAAAA | ACA  | CAATACATAT | CAACTTCGCT | ATTTTTTAA  | TAATTGCAAA | TATTATCTAC | 60  |
|---------|------|------------|------------|------------|------------|------------|-----|
| AGCAGCG | CCA  | GTGCATCAAC | AGATATCTCT | ACTGTTGCAT | CTCCATTATT | TGAAGGAACT | 120 |
| GAAGGTT | GTT  | TTTTACTTTA | CGATGCATCC | ACAAACGCTG | AAATTGCTCA | ATTCAATAAA | 180 |
| GCAAAGT | 'GTG | CAACGCAAAT | GGCACCAGAT | TCAACTTTCA | AGATCGCATT | ATCACTTATG | 240 |
| GCATTTG | ATG  | CGGAAATAAT | AGATCAGAAA | ACCATATTCA | AATGGGATAA | AACCCCCAAA | 300 |
| GGAATGG | AGA  | TCTGGAACAG | CAATCATACA | CCAAAGACGT | GGATGCAATT | TTCTGTTGTT | 360 |
| TGGGTTI | CGC  | AAGAAATAAC | CCAAAAAATT | AGATTAAATA | AAATCAAGAA | TTATCTCAAA | 420 |
| GATTTTG | ATT  | ATGGAAATCA | AGACTTCTCT | GGAGATAAAG | AAAGAAACAA | CGGATTAACA | 480 |
| GAAGCAT | GGC  | TCGAAAGTAG | CTTAAAAATT | TCACCAGAAG | AACAAATTCA | ATTCCTGCGT | 540 |
| AAAATTA | ATTA | ATCACAATCT | CCCAGTTAAA | AACTCAGCCA | TAGAAAACAC | CATAGAGAAC | 600 |
| ATGTATO | TAC  | AAGATCTGGA | TAATAGTACA | AAACTGTATG | GGAAAACTGG | TGCAGGATTC | 660 |
| ACAGCAA | ATA  | GAACCTTACA | AAACGGATGG | TTTGAAGGGT | TTATTATAAG | CAAATCAGGA | 720 |
| CATAAAT | ATG  | TTTTTGTGTC | CGCACTTACA | GGAAACTTGG | GGTCGAATTT | AACATCAAGC | 780 |
| ATAAAAG | CCA  | AGAAAAATGC | GATCACCATT | CTAAACACAC | TAAATTTATA | A          | 831 |

- (2) INFORMATION FOR SEQ ID NO: 111:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 846 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

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| (xi) | SEQUENCE | DESCRIPTION: | SEQ | ID | NO: | 111: |
|------|----------|--------------|-----|----|-----|------|
|------|----------|--------------|-----|----|-----|------|

| TTGAAAAAGT | TAATATTTTT | AATTGTAATT | GCTTTAGTTT | TAAGTGCATG | TAATTCAAAC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| AGTTCACATG | CCAAAGAGTT | AAATGATTTA | GAAAAAAAAT | ATAATGCTCA | TATTGGTGTT | 120 |
| TATGCTTTAG | ATACTAAAAG | TGGTAAGGAA | GTAAAATTTA | ATTCAGATAA | GAGATTTGCC | 180 |
| TATGCTTCAA | CTTCAAAAGC | GATAAATAGT | GCTATTTTGT | TAGAACAAGT | ACCTTATAAT | 240 |
| AAGTTAAATA | AAAAAGTACA | TATTAACAAA | GATGATATAG | TTGCTTATTC | TCCTATTTTA | 300 |
| GAAAAATATG | TAGGAAAAGA | TATCACTTTA | AAAGCACTTA | TTGAGGCTTC | AATGACATAT | 360 |
| AGTGATAATA | CAGCAAACAA | TAAAATTATA | AAAGAAATCG | GTGGAATCAA | AAAAGTTAAA | 420 |
| CAACGTCTAA | AAGAACTAGG | AGATAAAGTA | ACAAATCCAG | TTAGATATGA | GATAGAATTA | 480 |
| AATTACTATT | CACCAAAGAG | CAAAAAAGAT | ACTTCAACAC | CTGCTGCTTT | CGGTAAGACT | 540 |
| TTAAATAAAC | TTATCGCAAA | TGGAAAATTA | AGCAAAGAAA | ACAAAAAATT | CTTACTTGAT | 600 |
| TTAATGTTAA | ATAATAAAAG | CGGAGATACT | TTAATTAAAG | ACGGTGTTCC | AAAAGACTAT | 660 |
| AAGGTTGCTG | ATAAAAGTGG | TCAAGCAATA | ACATATGCTT | CTAGAAATGA | TGTTGCTTTT | 720 |
| GTTTATCCTA | AGGGCCAATC | TGAACCTATT | GTTTTAGTCA | TTTTTACGAA | TAAAGACAAT | 780 |
| AAAAGTGATA | AGCCAAATGA | TAAGTTGATA | AGTGAAACCG | CCAAGAGTGT | AATGAAGGAA | 840 |
| TTTTAA     |            |            |            |            | L          | 846 |

## (2) INFORMATION FOR SEQ ID NO: 112:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 555 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

ATGTCCGCGA GCACCCCCC CATAACTCTT CGCCTCATGA CCGAGCGCGA CCTGCCGATG 60

CTCCATGACT GGCTCAACCG GCCGCACATC GTTGAGTGGT GGGGTGGCGA CGAAGAGCGA 120

CCGACTCTTG ATGAAGTGCT GGAACACTAC CTGCCCAGAG CGATGGCGGA AGAGTCCGTA 180

ACACCGTACA TCGCAATGCT GGGCGAGGAA CCGATCGGCT ATGCTCAGTC GTACGTCGCG 240

CTCGGAAGCG GTGATGGCTG GTGGGAAGAT GAAACTGATC CAGGAGTGCG AGGAATAGAC 300

CAGTCTCTGG CTGACCCGAC ACAGTTGAAC AAAGGCCTAG GAACAAGGCT TGTCCGCGCT 360

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| CTCGTTGAAC                    | TACTGTTCTC                          | GGACCCCACC | GTGACGAAGA | TTCAGACCGA | CCCGACTCCG | 420 |  |
|-------------------------------|-------------------------------------|------------|------------|------------|------------|-----|--|
| AACAACCATC                    | GAGCCATACG                          | CTGCTATGAG | AAGGCAGGAT | TCGTGCGGGA | GAAGATCATC | 480 |  |
| ACCACGCCTG                    | ACGGGCCGGC                          | GGTTTACATG | GTTCAAACAC | GACAAGCCTT | CGAGAGAAAG | 540 |  |
| CGCGGTGTTG                    | CCTAA                               |            |            |            |            | 555 |  |
| (2) INFORM                    | (2) INFORMATION FOR SEQ ID NO: 113: |            |            |            |            |     |  |
| (;) epotence cuadaced tertes. |                                     |            |            |            |            |     |  |

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 732 base pairs
  - '(B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEOUENCE DESCRIPTION: SEO ID NO: 113:

ATGAACCAGA AAAACCCTAA AGACACGCAA AATTTTATTA CTTCTAAAAA GCATGTAAAA 60 GAAATATTGA ATCACACGAA TATCAGTAAA CAAGACAACG TAATAGAAAT CGGATCAGGA 120 AAAGGACATT TTACCAAAGA GCTAGTCAAA ATGAGTCGAT CAGTTACTGC TATAGAAATT 180 GATGGAGGCT TATGTCAAGT GACTAAAGAA GCGGTAAACC CCTCTGAGAA TATAAAAGTG 240 ATTCAAACGG ATATTCTAAA ATTTTCCTTC CCAAAACATA TAAACTATAA GATATATGGT 300 AATATTCCTT ATAACATCAG TACGGATATT GTCAAAAGAA TTACCTTTGA AAGTCAGGCT 360 AAATATAGCT ATCTTATCGT TGAGAAGGGA TTTGCGAAAA GATTGCAAAA TCTGCAACGA 420 480 GCTTTGGGTT TACTATTAAT GGTGGAGATG GATATAAAAA TGCTCAAAAA AGTACCACCA 540 CTATATTTTC ATCCTAAGCC AAGTGTAGAC TCTGTATTGA TTGTTCTTGA ACGACATCAA CCATTGATTT CAAAGAAGGA CTACAAAAAG TATCGATCTT TTGTTTATAA GTGGGTAAAC 600 CGTGAATATC GTGTTCTTTT CACTAAAAAC CAATTCCGAC AGGCTTTGAA GCATGCAAAT 660 GTCACTAATA TTAATAAACT ATCGAAGGAA CAATTTCTTT CTATTTTCAA TAGTTACAAA 720 TTGTTTCACT AA 732

- (2) INFORMATION FOR SEQ ID NO: 114:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 738 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

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| (xi) | SEQUENCE | DESCRIPTION: | SEO | ID | NO: | 114: |
|------|----------|--------------|-----|----|-----|------|
|------|----------|--------------|-----|----|-----|------|

| ATGAACAAAA            | ATATAAAATA | TTCTCAAAAC | TTTTTAACGA | GTGAAAAAGT | ACTCAACCAA | 60  |
|-----------------------|------------|------------|------------|------------|------------|-----|
| АТААТААААС            | AATTGAATTT | AAAAGAAACC | GATACCGTTT | ACGAAATTGG | AACAGGTAAA | 120 |
| GGGCATTTAA            | CGACGAAACT | GGCTAAAATA | AGTAAACAGG | TAACGTCTAT | TGAATTAGAC | 180 |
| AGTCATCTAT            | TCAACTTATC | GTCAGAAAAA | TTAAAATCGA | ATACTCGTGT | CACTTTAATT | 240 |
| CACCAAGATA            | TTCTACAGTT | TCAATTCCCT | AACAAACAGA | GGTATAAAAT | TGTTGGGAAT | 300 |
| ATTCCTTACC            | ATTTAAGCAC | ACAAATTATT | AAAAAAGTGG | TTTTTGAAAG | CCATGCGTCT | 360 |
| GACATCTATC            | TGATTGTTGA | AGAAGGATTC | TACAAGCGTA | CCTTGGATAT | TCACCGAACA | 420 |
| CTAGGGTTGC            | TCTTGCACAC | TCAAGTCTCG | ATTCAGCAAT | TGCTTAAGCT | GCCAGCGGAA | 480 |
| TGCTTTCATC            | CTAAACCAAG | AGTAAACAGT | GTCTTAATAA | AACTTACCCG | CCATACCACA | 540 |
| GATGTTCCAG            | ATAAATATTG | GAAGCTATAT | ACGTACTTTG | TTTCAAAATG | GGTCAATCGA | 600 |
| GAATATCGTC            | AACTGTTTAC | TAAAAATCAG | TTTCATCAAG | CAATGAAACA | CGCCAAAGTA | 660 |
| AACAATTTAA            | GTACCGTTAC | TTATGAGCAA | GTATTGTCTA | TTTTTAATAG | TTATCTATTA | 720 |
| יייייא <i>א</i> רכבכא | CCAAATAA   | •          |            |            |            | 738 |

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## (2) INFORMATION FOR SEQ ID NO: 115:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 735 base pairs
  - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

| ATGAACGAGA | AAATATAAA  | ACACAGTCAA | AACTTTATTA | CTTCAAAACA | TAATATAGAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| AAAATAATGA | CAAATATAAG | ATTAAATGAA | CATGATAATA | TCTTTGAAAT | CGGCTCAGGA | 120 |
| AAAGGGCATT | TTACCCTTGA | ATTAGTACAG | AGGTGTAATT | TCGTAACTGC | CATTGAAATA | 180 |
| GACCATAAAT | TATGCAAAAC | TACAGAAAAT | AAACTTGTTG | ATCACGATAA | TTTCCAAGTT | 240 |
| TTAAACAAGG | ATATATTGCA | GTTTAAATTŢ | CCTAAAAACC | AATCCTATAA | AATATTTGGT | 300 |
| AATATACCTT | ATAACATAAG | TACGGATATA | ATACGCAAAA | TTGTTTTTGA | TAGTATAGCT | 360 |
| GATGAGATTT | ATTTAATCGT | GGAATACGGG | TTTGCTAAAA | GATTATTAAA | TACAAAACGC | 420 |
| TCATTGGCAT | TATTTTTAAT | GGCAGAAGTT | GATATTTCTA | TATTAAGTAT | GGTTCCAAGA | 480 |

| TTATTTAATA | AGTAA      |            |            |            |            | 735 |
|------------|------------|------------|------------|------------|------------|-----|
| ATTGACGATT | TAAACAATAT | TAGCTTTGAA | CAATTCTTAT | CTCTTTTCAA | TAGCTATAAA | 720 |
| AAAGAATACA | AGAAAATATT | TACAAAAAAT | CAATTTAACA | ATTCCTTAAA | ACATGCAGGA | 660 |
| TCAAGAATAT | CACACAAAGA | TAAACAGAAG | TATAATTATT | TCGTTATGAA | ATGGGTTAAC | 600 |
| GAATATTTTC | ATCCTAAACC | TAGAGTGAAT | AGCTCACTTA | TCAGATTAAA | TAGAAAAAA  | 540 |

## (2) INFORMATION FOR SEQ ID NO: 116:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1029 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

| ATGAATAAAA | TAAAAGTCGC | AATTATCTTC | GGCGGTTGCT | CGGAGGAACA | TGATGTGTCG | 60   |
|------------|------------|------------|------------|------------|------------|------|
| GTAAAATCCG | CAATAGAAAT | TGCTGCGAAC | ATTAATACTG | AAAAATTCGA | TCCGCACTAC | 120  |
| ATCGGAATTA | CAAAAAACGG | CGTATGGAAG | CTATGCAAGA | AGCCATGTAC | GGAATGGGAA | 180  |
| GCCGATAGTC | TCCCCGCCAT | ATTCTCCCCG | GATAGGAAAA | CGCATGGTCT | GCTTGTCATG | 240  |
| AAAGAAAGAG | AATACGAAAC | TCGGCGTATT | GACGTGGCTT | TCCCGGTTTT | GCATGGCAAA | 300  |
| TGCGGGGAGG | ATGGTGCGAT | ACAGGGTCTG | TTTGAATTGT | CTGGTATCCC | CTATGTAGGC | 360  |
| TGCGATATTC | AAAGCTCCGC | AGCTTGCATG | GACAAATCAC | TGGCCTACAT | TCTTACAAAA | 420  |
| AATGCGGGCA | TCGCCGTCCC | CGAATTTCAA | ATGATTGAAA | AAGGTGACAA | ACCGGAGGCG | 480  |
| AGGACGCTTA | CCTACCCTGT | CTTTGTGAAG | CCGGCACGGT | CAGGTTCGTC | CTTTGGCGTA | 540  |
| ACCAAAGTAA | ACAGTACGGA | AGAACTAAAC | GCTGCGATAG | AAGCAGCAGG | ACAATATGAT | 600  |
| GGAAAAATCT | TAATTGAGCA | AGCGATTTCG | GGCTGTGAGG | TCGGCTGCGC | GGTCATGGGA | 660  |
| AACGAGGATG | ATTTGATTGT | CGGCGAAGTG | GATCAAATCC | GGTTGAGCCA | CGGTATCTTC | 720  |
| CGCATCCATC | AGGAAAACGA | GCCGGAAAAA | GGCTCAGAGA | ATGCGATGAT | TATCGTTCCA | 780  |
| GCAGACATTC | CGGTCGAGGA | ACGAAATCGG | GTGCAAGAAA | CGGCAAAGAA | AGTATATCGG | 840  |
| GTGCTTGGAT | GCAGAGGGCT | TGCTCGTGTT | GATCTTTTTT | TGCAGGAGGA | TGGCGGCATC | 900  |
| GTTCTAAACG | AGGTCAATAC | CCTGCCCGGT | TTTACATCGT | ACAGCCGCTA | TCCACGCATG | 960  |
| GCGGCTGCCG | CAGGAATCAC | GCTTCCCGCA | CTAATTGACA | GCCTGATTAC | ATTGGCGATA | 1020 |

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GAGAGGTGA 1029

#### (2) INFORMATION FOR SEQ ID NO: 117:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1031 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

ATGAAAAAA TTGCCGTTTT ATTTGGAGGG AATTCTCCAG AATACTCAGT GTCACTAACC 60 TCAGCAGCAA GTGTGATCCA AGCTATTGAC CCGCTGAAAT ATGAAGTAAT GACCATTGGC 120 ATCGCACCAA CAATGGATTG GTATTGGTAT CAAGGAAACC TCGCGAATGT TCGCAATGAT 180 ACTTGGCTAG AAGATCACAA AAACTGTCAC CAGCTGACTT TTTCTAGCCA AGGATTTATA 240 TTAGGAGAAA AACGAATCGT CCCTGATGTC CTCTTTCCAG TCTTGCATGG GAAGTATGGC 300 GAGGATGGCT GTATCCAAGG ACTGCTTGAA CTAATGAACC TGCCTTATGT TGGTTGCCAT 360 🔻 GTCGCTGCCT CCGCATTATG TATGAACAAA TGGCTCTTGC ATCAACTTGC TGATACCATG 420 GGAATCGCTA GTGCTCCCAC TTTGCTTTTA TCCCGCTATG AAAACGATCC TGCCACAATC 480 GATCGTTTTA TTCAAGACCA TGGATTCCCG ATCTTTATCA AGCCGAATGA AGCCGGTTCT 540 TCAAAAGGGA TCACAAAAGT AACTGACAAA ACAGCGCTCC AATCTGCATT AACGACTGCT 600 TTTGCTTACG GTTCTACTGT GTTGATCCAA AAGGCGATAG CGGGTATTGA AATTGGCTGC 660 GGCATCTTAG GAAATGAGCA ATTGACGATT GGTGCTTGTG ATGCGATTTC TCTTGTCGAC 720 GGTTTTTTTG ATTTTGAAGA GAAATACCAA TTAATCAGCG CCACGATCAC TGTCCCAGCA 780 CCATTGCCTC TCGCGCTTGA ATCACAGATC AAGGAGCAGG CACAGCTGCT TTATCGAAAC 840 TTGGGATTGA CGGGTCTGGC TCGAATCGAT TTTTTCGTCA CCAATCAAGG AGCGATTTAT 900 TTAAACGAAA TCAACACCAT GCCGGGATTT ACTGGGCACT CCCGCTACCC AGCTATGATG 960 GCGGAAGTCG GGTTATCCTA CGAAATATTA GTAGAGCAAT TGATTGCACT GGCAGAGGAG 1020 GACAAACGAT G 1031

1

### (2) INFORMATION FOR SEQ ID NO: 118:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 809 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double

| /D) | TOPOLOGY · | 1:     |
|-----|------------|--------|
| (T) | TOPOLOGY + | linear |

- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Abiotrophia adiacens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

TGGTGCTATC TTAGTAGTAT CTGCAGCTGA TGGTCCAATG CCTCAAACAC GTGAACACAT 60 CTTATTATCA CGTCAAGTAG GTGTTCCTTA CATCGTTGTA TTCTTAAACA AAGTTGACAT 120 GGTTGACGAT GAAGAATTAT TAGAATTAGT AGAAATGGAA GTTCGTGACT TATTATCAGA 180 ATACGATTTC CCAGGCGATG ACACTCCAGT TGTTGCAGGT TCTGCTTTAC GCGCTTTAGA 240 AGGCGACGCT TCATACRAAG AAAAAATCTT AGAATTAATG GCTGCTGTTG ACGAATACAT 300 TCCAACTCCA GAACGYGACG TTGACAAACC ATTCATGATG CCAGTTGAAG ACGTGTTCTC 360 AATCACAGGT CGTGGTACTG TTGCTACAGG TCGTGTTGAA CGTGGACAAG TTCGTGTTGG 420 TGACGAAGTT GAAATCGTTG GTATTTCAGA AGAAACTTCA AAAACAACTG TAACTGGTGT 480 TGAAATGTTC CGTAAATTGT TAGACTACGC TGAAGCAGGG GATAACATTG GTACATTATT 540 ACGTGGTGTT ACACGTGACA ACATCGAACG TGGACAAGTT CTTGCTAAAC CAGGAACAAT 600 CACTCCACAT ACTAAATTCA AAGCTGAAGT TTACGTATTA ACTAAAGAAG AAGGTGGACG 660 TCATACTCCA TTCTTCTCTA ACTACCGTCC TCAATTCTAC TTCCGTACAA CAGACATCAC 720 TGGTGTTTGT GTGTTACCAG AAGGCGTTGA AATGGTAATG CCTGGTGATA ACGTAACTAT 780 GGAAGTTGAA TTAATTCACC CAGTAGCGA 809

- (2) INFORMATION FOR SEQ ID NO: 119:
  - (i) SEQUENCE CHARACTERISTICS:
    - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double

(A) LENGTH: 817 base pairs

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Abiotrophia defectiva
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:
- CGGCGCGATC CTCGTTGTAT CTGCTGCTGA CGGCCCAATG CCACAAACTC GTGAACACAT 60
  CCTCTTGTCT CGTCAAGTTG GTGTTCCTTA CATCGTAGTA TTCTTGAACA AAGTTGACAT 120

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| GGTTGACGAC | GAAGAATTGC | TCGAATTAGT | TGAAATGGAA | GTTCGTGACC | TCTTGTCTGA | 180 |
|------------|------------|------------|------------|------------|------------|-----|
| ATACGACTTC | CCAGGCGACG | ACACTCCAGT | TATCGCTGGT | TCAGCTTTGA | AAGCTTTAGA | 240 |
| AGGCGACGCT | AACTACGAAG | CTAAAGTTTT | AGAATTGATG | GAACAAGTTG | ATGCTTACAT | 300 |
| TCCAGAACCA | GAACGTGACA | CTGACAAGCC | ATTCATGATG | CCAGTCGAAG | ACGTATTCTC | 360 |
| TATCACTGGT | CGTGGTACTG | TTGCAACTGG | TCGTGTTGAA | CGTGGTCAAG | TTCGCGTTGG | 420 |
| TGACGAAGTT | GAAATCGTTG | GTATCGAAGA | AGAAACTTCT | AAGACTACCG | TTACCGGTGT | 480 |
| TGAAATGTTC | CGTAAGTTAT | TGGATTACGC | TGAAGCTGGG | GACAACGTTG | GTACCTTGTT | 540 |
| ACGTGGTGTA | ACTCGTGACC | AAATCCAACG | TGGTCAAGTA | TTATCTAAAC | CAGGTTCAAT | 600 |
| CACTCCGYAC | ACTAAGTTCG | AAGCTGAAGT | GTACGTATTG | TCTAAAGAAG | AAGGTGGTCG | 660 |
| TCACACTCCA | TTCTTCTCTA | ACTACCGTCC | ACAATTCTAC | TTCCGTACAA | CTGACGTAAC | 720 |
| TGGTGTTGTT | ACTTTACCAG | AAGGTACTGA | AATGGTTATG | CCAGGCGACA | ACGTACAAAT | 780 |
| GGTTGTTGAA | TTGATCCACC | CAATCGCGAT | CGAAGAA    |            | r          | 817 |
|            |            |            |            |            |            |     |

#### (2) INFORMATION FOR SEQ ID NO: 120:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 754 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Candida albicans
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

CTCTGTCAAA TGGGACAAAA ACAGATTTGA AGAAATCATC AAGGAAACCT CCAACTTCGT 60 CAAGAAGGTT GGTTACAACC CAAAGACTGT TCCATTCGTT CCAATCTCTG GTTGGAATGG 120 TGACAACWTG ATTGAASCAT CCACCAACTG TCCATGGTAC AAGGGTTGGG AAAAGGAAAC 180 CAAATCCGGT AAAGTTACTG GTAAGACCTT GTTAGAAGCT ATTGACGCTA TTGAACCACC 240 AACCAGACCA ACCGACAAAC CATTGAGATT GCCATTRCAA GATGTTTACA AGATCGGTGG 300 TATTGGTACT GTGCCAGTCG GTAGAGTTGA AACTGGTATC ATCAAAGCCG GTATGGTWGT 360 TACTTTCGCC CCAGCTGGTG TTACCACTGA AGTCAARTCC GTTGAAATGC ATCACGAACA 420 ATTGGCTGAA GGTGTTCCAG GTGACAATGT TRGTTTCAAC GTTAAGAACR TTTCCGTTAA 480 AGAAATTAGA AGAGGTAACG TTTGTGGTGA CTCCAAGAAC GATCCACCAA AGGGTTGTGA 540

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| CTCTTTCAAT | GCCCAAGTCA | TTGTTTTGAA | CCATCCAGGT | CAAATCTCTG | CTGGTTACTC | 600 |
|------------|------------|------------|------------|------------|------------|-----|
| TCCAGTCTTG | GATTGTCACR | CTGCCCACAT | TGCTTGTAAA | TTCGACRCTT | TGGTTGAAAA | 660 |
| GATTGACAGA | AGAACTGGTA | AGRAATTGGA | AGAAAATCCA | AAATTCGTCA | AATCCGGTGA | 720 |
| TGCTGCTATC | GTCAAGATGG | TCCCAACCAA | ACCA       |            |            | 754 |

- (2) INFORMATION FOR SEQ ID NO: 121:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 753 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Candida glabrata
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

TCTGTCAAGT GGGATGAATC CAGATTCGCT GAAATCGTTA AGGAAACCTC CAACTTCATC 60 AAGAAGGTCG GTTACAACCC AAAGACTGTT CCATTCGTCC CAATCTCTGG TTGGAACGGT 120 GACAACATGA TTGAAGCCAC CACCAACGCT TCCTGGTACA AGGGTTGGGA AAAGGAAACC 180 . . AAGGCTGGTG TCGTCAAGGG TAAGACCTTG TTGGAAGCCA TTGACGCTAT CGAACCACCA 240 ACCAGACCAA CTGACAAGCC ATTGAGATTG CCATTGCAAG ATGTCTACAA GATCGGTGGT 300 ATCGGTACGG TGCCAGTCGG TAGAGTCGAA ACCGGTGTCA TCAAGCCAGG TATGGTTGTT 360 ACCTTCGCCC CAGCTGGTGT TACCACTGAA GTCAAGTCCG TTGAAATGCA CCACGAACAA 420 TTGACTGAAG GTTTGCCAGG TGACAACGTT GGTTTCAACG TTAAGAACGT TTCCGTTAAG 480 GAAATCAGAA GAGGTAATGT CTGTGGTGAC TCCAAGAACG ACCCACCAAA GGCTGCTGCT 540 TCTTTCAACG CTACCGTCAT TGTCTTGAAC CACCCAGGTC AAATCTCTGC TGGTTACTCT 600 CCAGTTTTGG ACTGTCACAC CGCCCACATT GCTTGTAAGT TCGAAGAATT GTTGGAAAAG 660 AACGACAGAA GATCCGGTAA GAAGTTGGAA GACTCTCCAA AGTTCTTGAA GTCCGGTGAC 720 753 GCTGCTTTGG TTAAGTTCGT TCCATCCAAG CCA

- (2) INFORMATION FOR SEQ ID NO: 122:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 752 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear

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| (ii)          | MOLECULE | TYPE: | DNA | (genomic)   |
|---------------|----------|-------|-----|-------------|
| \ <b>-</b> -/ |          |       |     | 13010111201 |

#### (vi) ORIGINAL SOURCE:

(A) ORGANISM: Candida krusei

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

| CCGTTAAGTG | GGATGAAAAC | AGATTTGAAG | AAATTGTCAA | GGAAACCCAA | AACTTCATCA | 6   |
|------------|------------|------------|------------|------------|------------|-----|
| AGAAGGTTGG | TTACAACCCA | AAGACTGTTC | CATTCGTTCC | AATCTCTGGT | TGGAATGGTG | 120 |
| ACAACATGAT | TGAAGCATCC | ACCAACTGTC | CATGGTACAA | GGGTTGGACT | AAGGAAACCA | 180 |
| AGGCAGGTGT | TGTTAAGGGT | AAGACCTTAT | TAGAAGCAAT | CGATGCTATT | GAACCACCTG | 240 |
| TCAGACCAAC | CGAAAAGCCA | TTAAGATTAC | CATTACAAGA | TGTTTACAAG | ATTGGTGGTA | 300 |
| TTGGTACTGT | GCCAGTCGGT | AGAGTCGAAA | CCGGTGTCAT | TAAGCCAGGT | ATGGTTGTCA | 360 |
| CTTTTGCTCC | AGCAGGTGTC | ACCACCGAAG | TCAAATCCGT | TGAAATGCAC | CATGAACAAT | 420 |
| TAGAACAAGG | TGTTCCAGGT | GATAACGTTG | GTTTCAACGT | TAAGAACGTY | TCTGTCAAGG | 480 |
| ATATCAAGAG | AGGTAACGTT | TGTGGTGACT | CCAAGAACGA | CCCACCAATG | GGTGCAGCTT | 540 |
| CTTTCAATGC | TCAAGTCATT | GTCTTGAACC | ACCCTGGTCA | AATTTCCGCT | GGTTACTCTC | 600 |
| CAGTCTTGGA | TTGTCACACT | GCCCACATTG | CATGTAAGTT | CGACGAATTA | ATCGAAAAGA | 660 |
| TTGACAGAAG | AACTGGTAAG | TCTGTTGAAG | ACCATCCAAA | GTCYGTCAAG | TCTGGTGATG | 720 |
| CACCTATCCT | CAACATCCTC | CCAACCAACC | CA         |            |            | 751 |

#### (2) INFORMATION FOR SEQ ID NO: 123:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 754 base pairs
  - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Candida parapsilosis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

CTCAGTCAAA TGGGACAAGA RCAGATACGA AGAAATTGTC AAGGAAACTT CCAACTTCGT 60

CAAGAAGGTT GGTTACAACC CTAAAGCTGT CCCATTCGTC CCAATCTCTG GTTGGAACGG 120

TGACAATATG ATTGAACCAT CAACCAACTG TCCATGGTAC AAGGGTTGGG AAAAGGAAAC 180

TAAAGCTGGT AAGGTTACCG GTAAGACCTT GTTGGAAGCT ATCGATGCTA TCGARCCACC 240

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| AACCAGACCA | ACTGACAAGC | CATTGAGATT | GCCATTGCAA | GATGTCTACA | AGATTGGTGG | 300 |
|------------|------------|------------|------------|------------|------------|-----|
| TATTGGAACT | GTGCCAGTTG | GTAGAGTTGA | AACCGGTATC | ATCAAGGCTG | GTATGGTTGT | 360 |
| TACTTTTGCC | CCAGCTGGTG | TTACCACTGA | AGTCAAGTCC | GTTGAAATGC | ACCACGAACA | 420 |
| ATTGACTGAA | GGTGTCCCAG | GTGACAATGT | TGGTTTCAAC | GTCAAGAACG | TTTCAGTTAA | 480 |
| GGAAATCAGA | AGAGGTAACG | TYTGTGGTGA | CTCCAAGAAC | GATCCACCAA | AGGGATGTGA | 540 |
| YTCCTTCAAT | GCTCAAGTTA | TTGTCTTGAA | CCACCCAGGT | CAAATCTCTG | CTGGTTACTC | 600 |
| ACCAGTCTTG | GATTGTCACA | CTGCCCACAT | TGCTTGTAAA | TTCGACACTT | TGATTGAAAA | 660 |
| GATTGACAGA | AGAACCGGTA | AGAAATTGGA | AGWTGAACCA | AAATTCATCA | AGTCCGGTGA | 720 |
| TGCTGCYATC | GTCAAGATGG | TCCCAACCAA | GCCA       |            |            | 754 |

- (2) INFORMATION FOR SEQ ID NO: 124:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 753 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Candida tropicalis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

| TCTGTTAAAT | GGGACAARAA | CAGATTTGAA | GAAATTATCA | AGGAAACYTC | TAACTTCGTC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| AAGAAGGTTG | GTTACAACCC | TAAGGCTGTT | CCATTCGTTC | CAATCTCWGG | TTGGAATGGT | 120 |
| GACAACATGA | TTGAAGCTTC | TACCAACTGT | CCATGGTACA | AGGGTTGGGA | AAAAGAAACC | 180 |
| AAGGCTGGTA | AGGTTACCGG | TAAGACTTTG | TTGGAAGCCA | TTGATGCTAT | TGAACCACCT | 240 |
| TCAAGACCAA | CTGACAAGCC | ATTGAGATTG | CCATTGCAAG | ATGTTTACAA | GATTGGTGGT | 300 |
| ATTGGTACTG | TGCCAGTCGG | TAGAGTTGAA | ACTGGTGTCA | TCAAAGCCGG | TATGGTTGTT | 360 |
| ACTTTYGCCC | CAGCTGGTGT | TACCACTGAA | GTCAAATCCG | TYGAAATGCA | CCACGAACAA | 420 |
| TTGGCTGAAG | GTGTCCCAGG | TGACAATGTT | GGTTTCAACG | TTAAGAACGT | TTCTGTTAAA | 480 |
| GAAATTAGAA | GAGGTAACGT | TTGTGGTGAC | TCCAAGAACG | ATCCACCAAA | GGGTTGTGAC | 540 |
| TCTTTCAACG | CTCAAGTTAT | TGTCTTGAAC | CACCCAGGTC | AAATYTCTGC | TGGTTACTCT | 600 |
| CCAGTCTTGG | ATTGTCACAC | TGCTCATATT | GCTTGTAAAT | TCGACACCTT | GGTTGAAAAG | 660 |
| ATTGACAGAA | GAACTGGTAA | GAAATTGGAA | GAAAATCCAA | AATTCGTCAA | ATCCGGTGAT | 720 |

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# GCTGCTATTG TCAAGATGGT TCCAACCAAA CCA 753 (2) INFORMATION FOR SEQ ID NO: 125: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 814 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic) (vi) ORIGINAL SOURCE: (A) ORGANISM: Corynebacterium accolens (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125: CGGCGCTATC CTGGTTGTTG CTGCAACCGA TGGCCCGATG CCGCAGACCC GCGAGCACGT 60 TCTGCTTGCT CGCCAGGTTG GCGTTCCTTA CATCCTCGTT GCACTGAACA AGTGCGACAT 120 GGTTGATGAT GAGGAAATCA TCGAGCTCGT GGAGATGGAG ATCTCCGAGC TGCTCGCAGA 180 GCAGGACTAC GATGAGGAAG CTCCTATCGT TCACATCTCC GCTCTGAAGG CACTCGAGGG 240 300 🗼 TGACGAGAAG TGGGTACAGT CCATCGTTGA CCTGATGGAT GCCTGCGACA ACTCCATCCC 360 TGATCCGGAG CGCGCTACCG ATCAGCCGTT CTTGATGCCT ATCGAGGACA TCTTCACCAT TACCGGCCGC GGTACCGTTG TTACCGGCCG TGTTGAGCGT GGTCGTCTGA ACGTCAACGA 420 GGACGTTGAG ATCATCGGTA TCCAGGAGAA GTCCCAGAAC ACCACCGTTA CCGGTATCGA 480 GATGTTCCGC AAGATGATGG ACTACACCGA GGCTGGCGAC AACTGTGGTC TGCTTCTGCG 540 TGGTACCAAG CGTGAGGACG TTGAGCGTGG CCAGGTTGTT ATCAAGCCGG GCGCTTACAC 600 CCCTCACACC AAGTTCGAGG GTTCCGTCTA CGTCCTGAAG AAGGAAGAGG GCGGCCGCCA 660 CACCCCGYTC ATGAACAACT ACCGTCCTCA GTTCTACTTC CGCACCACCG ACGTTACCGG 720

(2) INFORMATION FOR SEQ ID NO: 126:

TGTTGAGCTC ATCCAGCCTG TTGCTATGGA CGAG

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 814 base pairs

TGTTGTGAAC CTGCCTGAGG GCACCGAGAT GGTTATGCCT GGCGACAACG TTGAGATGTC

- (B) TYPE: nucleic acid
  (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

780

814

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## (A) ORGANISM: Corynebacterium diphteriae

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

| CGGCGCAATC | CTCGTTGTTG | CTGCCACCGA | CGGCCCAATG | CCTCAGACCC | GTGAGCACGT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| TCTGCTCGCT | CGCCAGGTCG | GCGTTCCTTA | CATCCTCGTT | GCTCTGAACA | AGTGCGACAT | 120 |
| GGTTGATGAT | GAGGAAATCA | TCGAGCTCGT | CGAGATGGAG | ATCCRTGAGC | TGCTCGCTGA | 180 |
| GCAGGATTAC | GACGAAGAGG | CTCCAATCAT | CCACATCTCC | GCACTGAAGG | CTCTTGAGGG | 240 |
| CGACGAGAAG | TGGACCCAGT | CCATCATCGA | CCTCATGCAG | GCTTGCKATG | ATTCCATCCC | 300 |
| AGACCCAGAG | CGTGAGACCG | ACAAGCCATT | CCTCATGCCT | ATCGAGGACA | TCTTCACCAT | 360 |
| CACCGGCCGC | GGTACCGTTG | TTACCGGCCG | TGTTGAGCGT | GGCTCCCTGA | AGGTCAACGA | 420 |
| GGACGTCGAG | ATCATCGGTA | TCCGCGAGAA | KGCTACCACC | ACCACCGTTA | CCGGTATCGA | 480 |
| GATGTTCCGT | AAGCTTCTCG | ACTACACCGA | GGCTGGCGAC | AACTGTGGTC | TGCTTCTCCG | 540 |
| TGGCGTTAAG | CGCGAAGACG | TTGAGCGTGG | CCAGGTTGTT | GTTAAGCCAG | GCGCTTACAC | 600 |
| CCCTCACACC | GAGTTCGAGG | GCTCTGTCTA | CGTTCTGTCC | AAGGACGAGG | GTGGCCGCCA | 660 |
| CACCCCATTC | TTCGACAACT | ACCGCCCACA | GTTCTACTTC | CGCACCACCG | ACGTTACCGG | 720 |
| TGTTGTGAAG | CTTCCTGAGG | GCACCGAGAT | GGTCATGCCT | GGCGACAACG | TCGACATGTC | 780 |
| CGTCACCCTG | ATCCAGCCTG | TCGCTATGGA | TGAG       |            |            | 814 |

### (2) INFORMATION FOR SEQ ID NO: 127:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 814 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Corynebacterium genitalium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

CGGCGCCATC CTGGTTGTTG CTGCAACCGA TGGCCCGATG CCGCAGACCC GTGAGCACGT 60

TCTGCTGGCT CGCCAGGTTG GCGTTCCGTA CATCCTAGTT GCACTGAACA AGTGCGACAT 120

GGTTGATGAT GAGGAGCTGC TGGAGCTCGT CGAGATGGAG GTCCGCGAGC TGCTGGCTGA 180

GCAGGACTTC GACGAGGAAG CACCTGTTGT TCACATCTCC GCACTGAAGG CCCTGGAGGG 240

CGACGAGAAG TGGGCTAAGC AGATCCTGGA GCTCATGGAG GCTTGCGACA ACTCCATCCC 300

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| GGATCCGGAG | CGCGAGACCG | ACAAGCCGTT | CCTGATGCCG | GTTGRGGACA | TCTTCACCAT | 360 |
|------------|------------|------------|------------|------------|------------|-----|
| TACCGGCCGC | GGTACCGTTG | TTACCGGCCG | TGTTGAGCGT | GGCGTCCTGA | ACCTGAACGA | 420 |
| CGAGGTCGAG | ATCCTGGGCA | TCCGCGAGAA | GTCCACCAAG | ACCACCGTTA | CCTCCATCGA | 480 |
| GATGTTCAAC | AAGCTGCTGG | ACACCGCAGA | GGCTGGCGAC | AACGCCGCAC | TGCTGCTGCG | 540 |
| TGGCCTGAAG | CGCGAAGATG | TTGAGCGTGG | TCAGATCGTT | GCTAAGCCGG | GCGAGTACAC | 600 |
| CCCGCACACC | GAGTTCGAGG | GCTCCGTCTA | CGTTCTGTCC | AAGGACGAGG | GTGGCCGCCA | 660 |
| CACCCCGTTC | TTCGACAACT | ACCGTCCGCA | GTTCTATTTC | CGCACCACCG | ACGTTACCGG | 720 |
| TGTTGTGAAG | CTGCCGGAGG | GCACCGAGAT | GGTTATGCCG | GGCGACAACG | TTGACATGTC | 780 |
| CGTCACCCTG | ATCCAGCCGG | TTGCTATGGA | CGAG       |            |            | 814 |

#### (2) INFORMATION FOR SEQ ID NO: 128:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 814 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Corynebacterium jeikeium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

CGGCGCCATC CTGGTTGTTG CCGCAACCGA TGGCCCGATG CCGCAGACCC GCGAGCACGT 60 TCTGCTGGCY CGCCAGGTTG GCGTTCCGTA CATCCTGGTT GCACTGAACA AGTGTGACAT 120 GGTTGACGAT GAGGAGCTGC TGGAGCTCGT CGAGATGGAG GTCCGCGAGC TGCTGGCTGA 180 GCAGGACTTC GACGAGGAAG CTCCGGTTGT TCACATCTCC GCACTGAAGG CCCTGGAGGG 240 CGACGAGAAG TGGGCTAACC AGATTCTCGA GCTGATGCAG GCTTGCGACG AGTCTATCCC 300 GGATCCGGAG CGCGAGACCG ACAAGCCGTT CCTGATGCCG GTTGWGGACA TCTTCACCAT 360 TACCGGTCGC GGTACCGTTG TTACCGGCCG TGTTGAGCGT GGCATCCTGA ACCTGAACGA 420 CGAGGTTGAG ATCCTGGGTA TCCGCGAGAA GTCCCAGAAG ACCACCGTTA CCTCCATCGA 480 GATGTTCAAC AAGCTGCTGG ACACCGCAGA GGCTGGCRAC AACGCTGCAC TGCTGCTGCG 540 TGGTCTGAAG CGCGAGGACG TTGAGCGTGG CCAGATCATC GCTAAGCCGG GCGAGTACAC 600 CCCGCACACC GAGTTCGAGG GCTCCGTCTA CGTTCTGTCC AAGGACGAGG GCGGCCGCCA 660 CACCCCGTTC TTCGACAACT ACCGTCCGCA GTTCTACTTC CGCACCACCG ACGTTACCGG 720

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| TGTTGTGAAG CTGCCTGAGG GCACCGAGAT GGTTATG  | CCG GGCGACAACG TYGACATGTC 78  |
|---|-------------------------------|
| CGTCACCCTG ATCCAGCCGG TTGCTATGGA CGAG   | 814                           |
| (2) INFORMATION FOR SEQ ID NO: 129:   |                               |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:         <ul> <li>(A) LENGTH: 748 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> </li> </ul> |                               |
| (ii) MOLECULE TYPE: DNA (genomic)   | •                             |
| (vi) ORIGINAL SOURCE:  (A) ORGANISM: Corynebacterium p  | pseudodiphteriticum           |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO  | : 129:                        |
| CGGCGCTATC TTGGTTGTTG CAGCTACCGA CGGCCCA  | ATG CCACAGACTC GCGAGCACGT 60  |
| TCTGCTGGCT CGCCAGGTTG GCGTTCCTTA CATCCTGC   | STT GCACTAAACA AGTGCGACAT 120 |
| GGTTGACGAC GAGGAAATCC TCGAGCTCGT CGAGATGC   | GAG ATCCGCGAAT TGCTGGCTGA 180 |
| CCAGGAATTC GACGAAGAAG CTCCAATCGT TCACATCT   | CC GCAGTCGGCG CCTTGGAAGG 240  |
| CGAAGAGAG TGGGTTAACG CCATCGTTGA ACTGATGO  | SAT GCTTGTGACG AGTCGATCCC 300 |
| TGATCCAGAC CGTGCTACCG ACAAGCCATT CCTGATGC   | CCT ATCGAGGACA TCTTCACCAT 360 |
| TACCGGTCGT GGCACCGTTG TTACGGGTCG TGTTGAGG   | GT GGTTCCCTGA AGGTCAACGA 420  |
| AGAAGTCGAG ATCATCGGCA TCAAGGAAAA GTCCCAGA   | AAG ACCACCATCA CCGGTATCGA 480 |
| AATGTTCCGC AAGATGCTGG ACTACACCGA GGCCGGCC   | SAC AACGCTGGTC TGCTGCTTCG 540 |
| CGGTACCAAG CGTGAAGACG TTGAGCGTGG ACAGGTTA   | ATC GTTGCTCCAG GTGCTTACAG 600 |
| CACCCACAG AAGTTCGAAG GTTCCGTCTA CGTTCTT   | CC AAGGACGAGG GCGGCCGCCA 660  |
| CACCCCGTTC TTCGACAACT ACCGTCCTCA GTTCTACT   | TTC CGCACCACCG ACGTTACCGG 720 |
| TGTTGTTACC CTGCCTGAGG GCACCGAG  | 748                           |
| (2) INFORMATION FOR SEQ ID NO: 130:   |                               |
| <ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 813 base pairs</li><li>(B) TYPE: nucleic acid</li><li>(C) STRANDEDNESS: double</li><li>(D) TOPOLOGY: linear</li></ul>                           |                               |

- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

| (A) | ORGANISM: | Corynebacterium | striatum |
|-----|-----------|-----------------|----------|
|-----|-----------|-----------------|----------|

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

| GGCGCTATCI | TGGTTGTTGC | TGCAACCGAT | GGCCCGRTGC | CGCAGACCCG | CGAGCACGTT | 60   |
|------------|------------|------------|------------|------------|------------|------|
| CTTCTGGCTC | GCCAGGTTGG | CGTTCCTTAC | ATCCTCGTTG | CACTGAACAA | GTGCGACATG | 120  |
| GTTGACGACG | AGGAAATTAT | CGAGCTCGTC | GAGATGGAGA | TCCGCGAACT | GCTCGCAGAG | 180  |
| CAGGACTACG | ATGAGGAAGC | TCCGATCGTT | CACATCTCTG | CTCTGAAGGC | TCTTGAGGGC | 240  |
| GRCGAGAAGT | GGGTACAGGC | TATCGTTGAC | CTGATGCAGG | CTTGCGATGA | CTCCATCCCG | 3.00 |
| GATCCGGAG  | GCGAGCTGGA | CAAGCCGTTC | CTGATGCCAA | TCGAGGACAT | CTTCACCATC | 360  |
| ACCGGCCGCG | GTACCGTTGT | TACTGGCCGT | GTTGAGCGTG | GCTCCCTGAA | CGTCAACGAG | 420  |
| GACGTTGAGA | TCATCGGTAT | CCAGGACARG | TCCATCTCCA | CCACCGTTAC | CGGTATCGAG | 480  |
| ATGYTCCGC  | AGATGATGGA | CTACACCGAG | GCTGGCGACA | ACTGTGGTCT | GCTTCTGCGT | 540  |
| GGTACCAAGO | GTGAAGAGGT | TGAGCGCGGC | CAGGTTGTTA | TTAAGCCGGG | CGCTTACACC | 600  |
| CCTCACACC  | AGTTCGAGGG | TTCCGTCTAC | GTCCTGAAGA | AGGAAGAGGG | CGGCCGCCAC | 660  |
| ACCCCGTTCA | TGGACAACTA | CCGTCCGCAG | TTCTACTTCC | GCACCACCGA | CGTTACCGGC | 720  |
| GTCATCAAGO | TGCCTGAGGG | CACCGAGATG | GTTATGCCTG | GCGACAACGT | CGAGATGTCY | 780  |
| GTCGAGCTGA | TCCAGCCGGT | CGCTATGGAC | GAG        |            |            | 813  |

## (2) INFORMATION FOR SEQ ID NO: 131:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Enterococcus avium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

| CGGAGCTATC | TTAGTAGTAT | CTGCTGCTGA | TGGCCCTATG | CCTCAAACTC | GTGAACACAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| CTTGTTATCT | CGTAACGTTG | GTGTTCCTTA | CATCGTTGTA | TTCTTAAACA | AAATGGATAT | 120 |
| GGTTGACGAT | GAAGAATTAC | TTGAATTAGT | TGAAATGGAA | GTTCGTGACT | TATTAACTGA | 180 |
| ATACGACTTC | CCAGGCGACG | ACACTCCAGT | TATCGCAGGT | TCAGCGTTGA | AAGCTTTAGA | 240 |
| AGGCGACGCT | TCATACGAAG | AAAAAATCTT | AGAATTAATG | GCTGCTGTTG | ACGAATATAT | 300 |

| CCCAACACCA | GTTCGTGATA | CTGACAAACC | ATTCATGATG | CCAGTCGAAG | ACGTATTCTC | 360 |
|------------|------------|------------|------------|------------|------------|-----|
| AATCACTGGT | CGTGGTACTG | TTGCAACTGG | TCGTGTTGAA | CGTGGACAAG | TTCGCGTTGG | 420 |
| TGACGAAGTT | GAAATCGTAG | GTATCGCTGA | CGAAACTGCT | AAAACAACTG | TTACAGGTGT | 480 |
| TGAAATGTTC | CGTAAATTGT | TAGACTACGC | TGAAGCAGGT | GACAACATCG | GTGCTTTGTT | 540 |
| ACGTGGTGTT | GCACGTGAAG | ATATCCAACG | TGGACAAGTA | TTGGCTAAAC | CAGCTTCAAT | 600 |
| CACTCCACAT | ACAAAATTCT | CTGCAGAAGT | TTATGTTCTA | ACTAAAGAAG | AAGGTGGACG | 660 |
| TCATACTCCA | TTCTTCACTA | ACTACCGTCC | TCAGTTCTAC | TTCCGTACAA | CTGACGTAAC | 720 |
| TGGTGTAGTT | GATCTACCAG | AAGGTACTGA | AATGGTWATG | CCTGGGGATA | ACGTAACTAT | 780 |
| GGAAGTTGAA | TTGATYCACC | CAATYGCGGT | AGAAGAC    |            |            | 817 |

- (2) INFORMATION FOR SEQ ID NO: 132:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Enterococcus faecalis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

| CGGAGCTATC | TTAGTAGTTT | CTGCTGCTGA | TGGTCCTATG | CCTCAAACAC | GTGAACATAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| CTTATTATCA | CGTAACGTTG | GTGTACCATA | CATCGTTGTA | TTCTTAAACA | AAATGGATAT | 120 |
| GGTTGATGAC | GAAGAATTAT | TAGAATTAGT | AGAAATGGAA | GTTCGTGACT | TATTATCAGA | 180 |
| ATACGATTTC | CCAGGCGATG | ATGTTCCAGT | TATCGCAGGT | TCTGCTTTGA | AAGCTTTAGA | 240 |
| AGGCGACGAG | TCTTATGAAG | AAAAAATCTT | AGAATTAATG | GCTGCAGTTG | ACGAATATAT | 300 |
| CCCAACTCCA | GAACGTGATA | CTGACAAACC | ATTCATGATG | CCAGTCGAAG | ACGTATTCTC | 360 |
| AATCACTGGA | CGTGGTACTG | TTGCTACAGG | ACGTGTTGAA | CGTGGTGAAG | TTCGCGTTGG | 420 |
| TGACGAAGTT | GAAATCGTTG | GTATTAAAGA | CGAAACATCT | AAAACAACYG | TTACAGGTGT | 480 |
| TGAAATGTTC | CGTAAATTAT | TAGACTACGC | TGAAGCAGGC | GACAACMTCG | GTGCTTTATT | 540 |
| ACGTGGTGTA | GCACGTGAAG | ATATCGAACG | TGGACAAGTA | TTAGCTAAAC | CAGCTACAAT | 600 |
| CACTCCACAC | ACAAAATTCA | AAGCTGAAGT | ATACGTATTA | TCAAAAGAAG | AAGGCGGACG | 660 |
| TCACACTCCA | TTCTTCACTA | ACTACCGTCC | TCAATTCTAC | TTCCGTACAA | CAGACGTTAC | 720 |

| TGGTGTTGTA GAATTGCCAG AAGGTACTGA AATGGTAATG CCTGGTGATA ACGTTGCTAT   | 78  |
|---|-----|
| GGACGTTGAA TTAATTCACC CAATCGCTAT CGAAGAC  | 81  |
| (2) INFORMATION FOR SEQ ID NO: 133:   |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 774 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> |     |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE:  (A) ORGANISM: Enterococcus faecium   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:  |     |
| CGGAGCTATC TTGGTAGTTT CTGCTGCTGA CGGCCCAATG CCTCAAACTC GTGAACACAT   | 60  |
| CCTATTGTCT CGTCAAGTTG GTGTTCCTTA CATCGTTGTA TTCTTGAACA AAGTAGACAT   | 120 |
| GGTTGATGAC GAAGAATTAC TAGAATTAGT TGAAATGGAA GTTCGTGACC TATTAACAGA   | 18( |
| ATACRAATTC CCTGGTGRCG ATGTTCCTGT AGTTGCTGGA TCAGCTTTGA AAGCTCTAGA   | 24( |
| AGGCGACGCT TCATACGAAG AAAAAATTCT TGAATTAATG GCTGCAGTTG ACGAATACAT   | 300 |
| CCCAACTCCA GAACGTGACA ACGACAAACC ATTCATGATG CCAGTTGAAG ACGTGTTCTC   | 360 |
| AATTACTGGA CGTGGTACTG TTGCTACAGG TCGTGTTGAA CGTGGACAAG TTCGCGTTGG   | 420 |
| TGACGAAGTT GAAGTTGTTG GTATTGCTGA AGAAACTTCA AAAACAACAG TTACTGGTGT   | 480 |
| TGAAATGTTC CGTAAATTGT TAGACYACGC TGAAGCTGGA GACRACATTG GTGCTTTACT   | 540 |
| ACGTGGTGTT GCACGTGAAG ACATCCAACG TGGACAAGTT TTAGCTAAAC CAGGTACAAT   | 600 |
| CACACCTCRT ACAAAATTCT CTGCAGAAGT ATACGTGTTG ACAAAAGAAG AAGGTGGACG   | 660 |
| TCATACTCCA TTCTTCACTA ACTACCGTCC ACAATTCTAC TTCCGTACAA CTGACGTAAC   | 720 |
| AGGTGTTGTT GAATTACCAG AAGGAACTGA AATGGTCATG CCCGGTGACA ACGT   | 774 |
| (2) INFORMATION FOR SEQ ID NO: 134:   |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 809 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> |     |

- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

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| (A) | ORGANISM: | Enterococcus | gallinarum |
|-----|-----------|--------------|------------|
|-----|-----------|--------------|------------|

#### (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

| CGGTGCGATC | TTAGTAGTAT | CTGCTGCTGA | CGGTCCTATG | CCTCAAACTC | GTGAACACAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| CTTGTTATCA | CGTAACGTTG | GCGTACCATA | CATCGTTGTT | TTCTTGAACA | AAATGGATAT | 120 |
| GGTTGAYGAC | GAAGAATTGC | TAGAATTAGT | TGAAATGGAA | GTTCGTGACC | TATTGTCTGA | 180 |
| ATATGACTTC | CCAGGCGACG | ATGTTCCTGT | AATCGCCGGT | TCTGCTTTGA | AAGCTCTTGA | 240 |
| AGGAGATCCT | TCATACGAAG | AAAAAATCAT | GGAATTGATG | GCTGCAGTTG | ACGAATACGT | 300 |
| TCCAACTCCA | GAACGTGATA | CTGACAAACC | ATTCATGATG | CCAGTCGAAG | ACGTATTCTC | 360 |
| AATCACTGGA | CGTGGTACTG | TTGCTACAGG | CCGTGTTGAA | CGTGGACAAG | TTCGCGTTGG | 420 |
| TGATGAAGTA | GAAATCGTTG | GTATTGCTGA | CGAAACTGCT | AAAACAACTG | TAACAGGTGT | 480 |
| TGAAATGTTC | CGTAAATTGT | TAGACTATGC | TGAAGCAGGG | GATAACATTG | GTGCATTGCT | 540 |
| ACGTGGGGTT | GCTCGTGAAG | ACATCCAACG | TGGACAAGTA | TTGGCTAAAG | CTGGTACAAT | 600 |
| CACACCTCAT | ACAAAATTCA | AAGCTGAAGT | TTATGTTTTG | ACAAAAGAAG | AAGGTGGACG | 660 |
| TCACACTCCA | TTCTTCACTA | ACTACCGTCC | TCAGTTCTAC | TTCCGTACAA | CTGACGTAAC | 720 |
| TGGTGTTGTT | GAATTACCAG | AAGGAACTGA | AATGGTGATG | CCTGGCGACA | ACGTGACCAT | 780 |
| CGACGTTGAA | TTGATRCACC | CAATCGCTC  |            | •          |            | 809 |

### (2) INFORMATION FOR SEQ ID NO: 135:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 823 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Gardnerella vaginalis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

TGGCGCAATC CTCGTGGTTG CTGCTACCGA CGGTCCAATG GCTCAGACCC GTGAACACGT 60

CTTGCTTGCT AAGCAGGTCG GCGTTCCAAA AATTCTTGTT GCTTTGAACA AGTGCGATAT 120

GGTTGACGAC GAAGAGCTTA TCGATCTCGT TGAAGAAGAG GTCCGTGACC TCCTCGAAGA 180

AAACGGCTTC GATCGCGATT GCCCAGTCYT CCGTACTTCC GCTTACGGCG CTTTGCATGA 240

TGACGCTCCA GACCACGACA AGTGGGTAGA GACCGTCAAG GAACTCATGA AGGCTGTTGA 300

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| CGAGTACATC | CCAACCCCAA | CTCACGATCT | TGACAAGCCA | TTCTTGATGC | CAATCGAAGA | 360 |
|------------|------------|------------|------------|------------|------------|-----|
| TGTGTTCACC | ATCTCCGGTC | GTGGTYCCGT | TGTCACCGGT | CGTGTTGAGC | GTGGTAAGCT | 420 |
| CCCAATCAAC | ACCCCAGTTG | AGATCGTTGG | TTTGCGCGAT | ACCCAGACCA | CCACCGTCAC | 480 |
| CTCTATCGAG | ACCTTCCACA | AGCAGATGGA | TGAGGCAGAG | GCTGGCGATA | ACACTGGTCT | 540 |
| TCTTCTCCGC | GGTATCAACC | GTACCGACGT | TGAGCGTGGT | CAGGTTGTGG | CTGCTCCAGG | 600 |
| TTCTGTGACT | CCACACACCA | AGTTCGAAGG | CGAAGTTTAC | GTCTTGACCA | AGGACGAAGG | 660 |
| TGGCCGTCAC | TCGCCATTCT | TCTCCAACTA | CCGTCCACAG | TTCTACTTCC | GTACCACCGA | 720 |
| TGTTACTGGC | GTTATCACCT | TGCCAGACGG | CATCGAAATG | GTTCAGCCAG | GCGATCACGC | 780 |
| AACCTTCACT | GTTGAGTTGA | TCCAGGCTAT | CGCAATGGAA | GAG        |            | 823 |

#### (2) INFORMATION FOR SEQ ID NO: 136:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Listeria innocua
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACATAT 60 120 CTTACTTTCA CGTCAAGTTG GTGTTCCATA CATCGTTGTA TTCATGAACA AATGTGACAT 180 GGTTGACGAT GAAGAATTAC TAGAATTAGT TGAAATGGAA ATTCGTGATC TATTAACTGA ATATGAATTC CCTGGCGATG ACATTCCTGT AATCAAAGGT TCAGCTCTTA AAGCACTTCA 240 AGGTGAAGCT GACTGGGAAG CTAAAATTGA CGAGTTAATG GAAGCTGTAG ATTCTTACAT 300 TCCAACTCCA GAACGTGATA CTGACAAACC ATTCATGATG CCAGTTGAGG ATGTATTCTC 360 AATCACTGGT CGTGGAACAG TTGCAACTGG ACGTGTTGAA CGTGGACAAG TTAAAGTTGG 420 480 TGACGAAGTA GAAGTTATCG GTATTGAAGA AGAAAGCAAA AAAGTAGTAG TAACTGGAGT AGAAATGTTC CGTAAATTAC TAGACTACGC TGAAGCTGGC GACAACATTG GCGCACTTCT 540 ACGTGGTGTT GCTCGTGAAG ATATCCAACG TGGTCAAGTA TTAGCTAAAC CAGGTTCGAT 600 TACTCCACAC ACTAACTTCA AAGCTGAAAC TTATGTTTTA ACTAAAGAAG AAGGTGGACG 660 TCACACTCCA TTCTTCAACA ACTACCGCCC ACAATTCTAT TTCCGTACTA CTGACGTAAC 720

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| TGGTATTGTT ACACTTCCAG AAGGTACTGA AATGGTAATG CCTGGTGATA ACATTGAGCT   | 780 |
|---|-----|
| TGCAGTTGAA CTAATTGCAC CAATCGCTAT CGAAGAC  | 817 |
| (2) INFORMATION FOR SEQ ID NO: 137:   |     |
| <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 818 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: double</li> <li>(D) TOPOLOGY: linear</li> </ul> |     |
| (ii) MOLECULE TYPE: DNA (genomic)   |     |
| (vi) ORIGINAL SOURCE:<br>(A) ORGANISM: <i>Listeria ivanovii</i>   |     |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:  |     |
| CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGTCCAATG CCACAAACTC GTGAACATAT   | 60  |
| TCTTACTTTC ACGTCAAGTT GGTGTTCCAT ACATCGTTGT ATTCATGAAC AAATGTGACA   | 120 |
| TGGTTGACGA TGAAGAATTA CTTGAATTAG TTGAAATGGA AATTCGTGAT CTATTAACTG   | 180 |
| AATATGAATT CCCTGGCGAC GACATTCCTG TAATCAAAGG TTCAGCTCTT AAAGCACTTC   | 240 |
| AAGGTGAAGC TGATTGGGAA GCTAAAATTG ACGAGTTAAT GGAAGCTGTA GATTCTTACA   | 300 |
| TTCCAACTCC AGAACGTGAT ACTGACAAAC CATTCATGAT GCCAGTTGAG GATGTATTCT   | 360 |
| CAATCACTGG TCGTGGAACA GTTGCAACTG GACGTGTTGA ACGTGGACAA GTTAAAGTTG   | 420 |
| GTGACGAAGT AGAAGTTATC GGTATTGAAG AAGAAAGCAA AAAAGTAGTA GTAACTGGAG   | 480 |
| TAGAAATGTT CCGTAAATTA CTAGACTACG CTGAAGCTGG CGACAACATT GGCGCACTTC   | 540 |
| TACGTGGTGT TGCTCGTGAA GATATCCAAC GTGGTCAAGT ATTAGCTAAA CCAGGTTCGA   | 600 |
| TTACTCCACA TACTAACTTC AAAGCTGAAA CTTATGTTTT AACTAAAGAA GAAGGTGGAC   | 660 |
| GTCATACTCC ATTCTTCAAC AACTACCGCC CACAATTCTA TTTCCGTACT ACTGACGTAA   | 720 |
| CTGGTATTGT TACACTTCCA GAAGGTACTG AAATGGTAAT GCCTGGTGAT AACATTGAGC   | 780 |
| TTGCAGTTGA ACTAATTGCA CCAATCGCTA TCGAAGAC   | 818 |
| (2) INFORMATION FOR SEQ ID NO: 138:   |     |
| (i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 817 base pairs   |     |

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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| 4 | lari 1 | ORIGINAL | COTTOCE. |
|---|--------|----------|----------|
| ١ | VI     | OKIGINAL | SOURCE:  |

(A) ORGANISM: Listeria monocytogenes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:

| CGGAGCTATC | TTAGTAGTAT | CTGCTGCTGA | TGGCCCAATG | CCACAAACTC | GTGAACATAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| CTTACTTTCA | CGTCAAGTTG | GTGTTCCATA | CATCGTTGTA | TTCATGAACA | AATGTGACAT | 120 |
| GGTTGACGAT | GAAGAATTAC | TAGAATTAGT | TGAAATGGAA | ATTCGTGATC | TATTAACTGA | 180 |
| ATATGAATTC | CCTGGCGATG | ACATTCCTGT | AATCAAAGGT | TCAGCTCTTA | AAGCACTTCA | 240 |
| AGGTGAAGCT | GACTGGGAAG | CTAAAATTGA | CGAGTTAATG | GAAGCTGTAG | ATTCTTACAT | 300 |
| TCCAACTCCW | GAACGTGATA | CTGACAAACC | ATTCATGATG | CCAGTTGAGG | ATGTATTCTC | 360 |
| AATCACTGGT | CGTGGAACAG | TTGCAACTGG | ACGTGTTGAA | CGTGGACAAG | TTAAAGTTGG | 420 |
| TGACGAAGTA | GAAGTTATCG | GTATCGAAGA | AGAAAGCAAA | AAAGTAGTAG | TAACTGGAGT | 480 |
| AGAAATGTTC | CGTAAATTAC | TAGACTACGC | TGAAGCTGGC | GACAACATTG | GCGCACTTCT | 540 |
| ACGTGGTGTT | GCTCGTGAAG | ATATCCAACR | TGGTCAAGTA | TTAGCTAAAC | CAGGTTCGAT | 600 |
| TACTCCACAC | ACTAACTTCA | AAGCTGAAAC | TTATGTTTTA | ACTAAAGAAG | AAGGTGGACG | 660 |
| TCACACTCCA | TTCTTCAACA | ACTACCGÇCC | ACAATTCTAT | TTCCGTACTA | CTGACGTAAC | 720 |
| TGGTATTGTT | ACACTTCCAG | AAGGTACTGA | AATGGTAAYG | CCTGGTGATA | ACATTGAGCT | 780 |
| TGCAGTTGAA | СТААТТССАС | CAATCGCTAT | CGAAGAC    |            |            | 817 |

#### (2) INFORMATION FOR SEQ ID NO: 139:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Listeria seeligeri
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACATAT 60

CTTACTTTCA CGTCAAGTTG GTGTTCCATA CATCGTTGTA TTCATGAACA AATGTGACAT 120

GGTTGACGAT GAAGAATTAC TTGAATTAGT TGAAATGGAA ATTCGTGATC TATTAACTGA 180

ATATGAATTC CCTGGTGATG ACATTCCTGT AATCAAAGGT TCAGCTCTTA AAGCACTTCA 240

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| AGGTGAAGCT | GACTGGGAAG | CTAAAATTGA | CGAGTTAATG | GAAGCTGTAG | ATTCTTACAT | 300 |
|------------|------------|------------|------------|------------|------------|-----|
| TCCAACTCCA | GAACGTGATA | CTGACAAACC | ATTCATGATG | CCAGTTGAGG | ATGTATTCTC | 360 |
| AATCACTGGT | CGTGGAACTG | TTGCAACTGG | ACGTGTTGAA | CGTGGACAAG | TTAAAGTTGG | 420 |
| TGACGAAGTA | GAAGTTATCG | GTATTGAAGA | AGAAAGCAAA | AAAGTAATAG | TAACTGGAGT | 480 |
| AGAAATGTTC | CGTAAATTAC | TAGACTACGC | TGAAGCTGGC | GACAACATTG | GCGCACTTCT | 540 |
| ACGTGGTGTT | GCTCGTGAAG | ATATCCAACG | TGGTCAAGTA | TTAGCTAAAC | CAGGTTCGAT | 600 |
| TACTCCACAT | ACTAACTTCA | AAGCTGAAAC | TTATGTTTTA | ACTAAAGAAG | AAGGTGGACG | 660 |
| TCACACTCCA | TTCTTCAACA | ACTACCGCCC | ACAATTCTAT | TTCCGTACTA | CTGACGTAAC | 720 |
| TGGTATTGTT | ACACTTCCAG | AAGGTACTGA | AATGGTAATG | CCTGGTGATA | ACATTGAGCT | 780 |
| TGCAGTTGAA | CTAATTGCAC | CAATCGCTAT | CGAAGAC    |            |            | 817 |

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- (2) INFORMATION FOR SEQ ID NO: 140:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 814 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

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- (A) ORGANISM: Staphylococcus aureus
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:

CGGTGGTATC TTAGTAGTAT CTGCTGCTGA CGGTCCAATG CCACAAACTC GTGAACACAT TCTTTTATCA CGTAACGTTG GTGTACCAGC ATTAGTAGTA TTCTTAAACA AAGTTGACAT 120 180 GGTTGACGAT GAAGAATTAT TAGAATTAGT AGAAATGGAA GTTCGTGACT TATTAAGCGA ATATGACTTC CCAGGTGACG ATGTACCTGT AATCGCTGGT TCAGCATTAR AAGCTTTAGA 240 AGGCGATGCT CAATACGAAG AAAAAATCTT AGAATTARTG GAAGCTGTAG ATACTTACAT 300 TCCAACTCCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC 360 420 AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTTGG TGAAGAAGTT GAAATCATCG GTTTACATGA CACATCTAAA ACAACTGTTA CAGGTGTTGA 480 540 AATGTTCCGT AAATTATTAG ACTACGCTGA AGCTGGTGAC AACATTGGTG CATTATTACG 600 TGGTGTTGCT CGTGAAGACG TACAACGTGG TCAAGTATTA GCTGCTCCTG GTTCAATTAC ACCACATACT GAATTCAAAG CAGAAGTATA CGTATTATCA AAAGACGAAG GTGGACGTCA 660

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| CACTCCATTC | TTCTCAAACT | ATCGTCCACA | ATTCTATTTC | CGTACTACTG | ACGTAACTGG | 720 |
|------------|------------|------------|------------|------------|------------|-----|
| TGTTGTTCAC | TTACCAGAAG | GTACTGAAAT | GGTAATGCCT | GGTGATAACG | TTGAAATGAC | 780 |
| AGTAGAATTA | ATCGCTCCAA | TCGCGATTGA | AGAC       |            |            | 814 |

- (2) INFORMATION FOR SEQ ID NO: 141:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 814 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Staphylococcus epidermidis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

60 CGGCGGTATC TTAGTTGTAT CTGCTGCTGA CGGTCCAATG CCACAAACTC GTGAACACAT CTTATTATCA CGTAACGTTG GTGTACCAGC ATTAGTTGTA TTCTTAAACA AAGTTGACAT 120 180 GGTAGACGAC GAAGAATTAT TAGAATTAGT TGAAATGGAA GTTCGTGACT TATTAAGCGA ATATGACTTC CCAGGTGACG ATGTACCTGT AATCGCTGGT TCTGCATTAA AAGCATTAGA 240 . . AGGCGATGCT GAATACGAAC AAAAAATCTT AGACTTAATG CAAGCAGTTG ATGATTACAT 300 360 TCCAACTCCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTWGG 420 TGAAGAAGTT GAAATCATCG GTATGCACGA AACTTCTAAA ACAACTGTTA CTGGTGTAGA AATGTTCCGT AAATTATTAG ACTACGCTGA AGCTGGTGAC AACATCGGTG CTTTATTACG 540 600 TGGTGTTGCA CGTGAAGACG TACAACGTGG TCAAGTATTA GCTGCTCCTG GTTCTATTAC ACCACACACA AAATTCAAAG CTGAAGTATA CGTATTATCT AAAGATGAAG GTGGACGTCA 660 720 CACTCCATTC TTCACTAACT ATCGCCCACA ATTCTATTTC CRTACTACTG ACGTAACTGG 780 TGTTGTAAAC TTACCAGAAG GTACAGAAAT GGTTATGCCT GGCGACAACG TTGAAATGAC AGTTGAATTA ATCGCTCCAA TCGCTATCGA AGAC 814

- (2) INFORMATION FOR SEQ ID NO: 142:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear

| (ii) | MOLECULE | TYPE: | DNA | (genomic) |
|------|----------|-------|-----|-----------|
|------|----------|-------|-----|-----------|

#### (vi) ORIGINAL SOURCE:

(A) ORGANISM: Staphylococcus saprophyticus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACACAT 60 TCTTTTATCA CGTRACGTTG GTGYTCCAGC ATTAGTTGTA TTCTTAAACA AAGTTGACAT 120 GGTTGACGAY GAAGAATTAT TAGAATTRGT AGAAATGGAA GTTCGTGRCT TATTAAGCGA 180 ATATGACTTC CCAGGTGACG ATGTACCTGT AATCTCTGGT TCTGCATTAA AAGCTTTAGA 240 AGGCGACGCT GACTATGAGC AAAAAATCTT AGACTTAATG CAAGCTGTTG ATGACTYCAT 300 TCCAACACCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC 360 AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTCGG 420 TGAAGAAATC GARATCATCG GTATGCAAGA AGAATCAAGC AAAACAACTG TTACTGGTGT 480 AGAAATGTTC CGTAAATTAT TAGACTACGC TGAAGCTGGT GACAACATTG GTGCATTATT 540 ACGTGGTGTT TCACGTGATG ATGTACAACG TGGTCAAGTT TTAGCTGCTC CTGGTACTAT 600 660 CACACCACAT ACAAAATTCA AAGCGGATGT TTACGTTTTA TCTAAAGATG AAGGTGGTCG TCATACGCCA TTCTTCACTA ACTACCGCCC ACAATTCTAT TTCCGTACTA CTGACGTAAC 720 TGGTGTTGTT AACTTACCAG AAGGTACTGA AATGGTTATG CCTGGCGATA ACGTTGAAAT 780 817 GGATGTTGAA TTAATTTCTC CAATCGCTAT TGAAGAC

### (2) INFORMATION FOR SEQ ID NO: 143:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Staphylococcus simulans
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

CGGCGGTATC TTAGTAGTAT CTGCTGCAGA TGGTCCAATG CCACAAACTC GTGAACACAT 60

CTTATTATCA CGTAACGTTG GTGTACCAGC TTTAGTTGTA TTCTTAAACA AAGCTGACAT 120

GGTTGACGAC GAAGAATTAT TAGAATTAGT TGAAATGGAA GTTCGTGACT TATTATCTGA 180

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| ATACGACTTC | CCTGGTGACG | ATGTACCAGT | TATCGTTGGT | TCTGCATTAA | AAGCTTTAGA | 240 |
|------------|------------|------------|------------|------------|------------|-----|
| AGGCGACCCA | GAATACGAAC | AAAAAATCTT | AGACTTAATG | CAAGCTGTAG | ATGACTACAT | 300 |
| CCCAACTCCA | GAACGTGACT | CTGATAAACC | ATTCATGATG | CCAGTTGAGG | ACGTATTCTC | 360 |
| AATCACTGGT | CGTGGTACTG | TAGCAACAGG | CCGTGTTGAA | CGTGGTCAAA | TCAAAGTCGG | 420 |
| TGAAGAAGTT | GAAATCATCG | GTATCACTGA | AGAAAGCAAG | AAAACAACAG | TTACAGGTGT | 480 |
| AGAAATGTTC | CGTAAATTAT | TAGACTACGC | TGAAGCTGGT | GACAACATCG | GTGCTTTATT | 540 |
| ACGTGGTGTT | GCACGTGAAG | ACGTACAACG | TGGACAAGTA | TTAGCAGCTC | CTGGCTCTAT | 600 |
| TACTCCACAC | ACAAAATTCA | AAGCTGATGT | TTACGTTTTA | TCTAAAGAAG | AAGGTGGACG | 660 |
| TCATACTCCA | TTCTTCACTA | ACTACCGCCC | ACAATTCTAC | TTCCGTACTA | CTGACGTAAC | 720 |
| TGGCGTTGTT | CACTTACCAG | AAGGTACTGA | AATGGTTATG | CCTGGCGATA | ACGTAGAAAT | 780 |
| GACTGTTGAA | TTGATCGCTC | CAATCGCGAT | TGAAGAC    |            |            | 817 |

#### (2) INFORMATION FOR SEQ ID NO: 144:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus agalactiae
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:

CGGAGCTATC CTTGTAGTTG CTTCAACTGA TGGACCAATG CCACAAACTC GTGAGCACAT 60 CCTTCTTTCA CGTCAAGTTG GTGTTAAACA CCTTATCGTA TTCATGAACA AAGTTGACCT 120 TGTTGATGAT GAAGAATTGC TTGAATTGGT TGAAATGGAA ATTCGTGACC TTCTTTCAGA 180 ATACGACTTC CCAGGTGATG ACCTTCCAGT TATCCAAGGT TCAGCTCTTA AAGCACTTGA 240 AGGCGACGAA AAATACGAAG ACATCATCAT GGAATTGATG AGCACTGTTG ATGAGTACAT 300 TCCAGAACCA GAACGTGATA CTGACAAACC TTTACTTCTT CCAGTTGAAG ATGTATTCTC 360 AATCACTGGA CGTGGTACAG TTGCTTCAGG ACGTATCGAC CGTGGTACTG TTCGTGTCAA 420 CGACGAAGTT GAAATCGTTG GTATTAAAGA AGATATCCAA AAAGCAGTTG TTACTGGTGT 480 TGAAATGTTC CGTAAACAAC TTGACGAAGG TCTTGCAGGG GACAACGTTG GTGTTCTTCT 540 TCGTGGTGTT CAACGTGATG AAATCGAACG TGGTCAAGTT CTTGCTAAAC CAGGTTCAAT 600

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| CAACCCACAC | ACTAAATTTA | AAGGTGAAGT | TTACATCCTT | TCTAAAGAAG | AAGGTGGACG | 660 |
|------------|------------|------------|------------|------------|------------|-----|
| TCATACTCCA | TTCTTCAACA | ACTACCGTCC | ACAATTCTAC | TTCCGTACAA | CTGACGTAAC | 720 |
| AGGTTCAATC | GAACTTCCAG | CAGGAACAGA | AATGGTTATG | CCTGGTGATA | ACGTTACTAT | 780 |
| CGAAGTTGAA | TTGATTCACC | CAATCGCCGT | AGAACAA    |            |            | 817 |

- (2) INFORMATION FOR SEQ ID NO: 145:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Streptococcus pneumoniae
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:

| CGGAGCTATC | CTTGTAGTAG | CTTCAACTGA | CGGACCAATG | CCACAAACTC | GTGAGCACAT | 60 ·  |
|------------|------------|------------|------------|------------|------------|-------|
| CCTTCTTTCA | CGTCAGGTTG | GTGTTAAACA | CCTTATCGTC | TTCATGAACA | AAGTTGACTT | 120 , |
| GGTTGACGAC | GAAGAATTGC | TTGAATTGGT | TGAAATGGAA | ATCCGTGACC | TATTGTCAGA | 180 . |
| ATACGACTTC | CCAGGTGACG | ATCTTCCAGT | TATCCAAGGT | TCAGCACTTA | AAGCTCTTGA | 240   |
| AGGTGACTCT | AAATACGAAG | ACATCGTTAT | GGAATTGATG | AACACAGTTG | ATGAGTATAT | 300   |
| CCCAGAACCA | GAACGTGACA | CTGACAAACC | ATTGCTTCTT | CCAGTCGAGG | ACGTATTCTC | 360   |
| AATCACTGGA | CGTGGTACAG | TTGCTTCAGG | ACGTATCGAC | CGTGGTATCG | TTAAAGTCAA | 420   |
| CGACGAAATC | GAAATCGTTG | GTATCAAAGA | AGAAACTCRA | AAAGCAGTTG | TTACTGGTGT | 480   |
| TGAAATGTTC | CGTAAACAAC | TTGACGAAGG | TCTTGCTGGA | GATAACGTAG | GTGTCCTTCT | 540   |
| TCGTGGTGTT | CAACGTGATG | AAATCGAACG | TGGACAAGTT | ATCGCTAAAC | CAGGTTCAAT | 600   |
| CAACCCACAC | ACTAAATTCA | AAGGTGAAGT | CTACATCCTT | ACTAAAGAAG | AAGGTGGACG | 660   |
| TCACACTCCA | TTCTTCAACA | ACTACCGTCC | ACAATTCTAC | TTCCGTACTA | CTGACGTTAC | 720   |
| AGGTTCAATC | GAACTTCCAG | CAGGTACTGA | AATGGTAATG | CCTGGTGATA | ACGTGACAAT | 780   |
| CGACGTTGAG | TTGATTCACC | CAATCGCCGT | AGAACAA    |            |            | 817   |

- (2) INFORMATION FOR SEQ ID NO: 146:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid

| (C) | STRANDEDN | ESS: | double |
|-----|-----------|------|--------|
| (D) | TOPOLOGY: | line | ear    |

- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus salivarius
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

| CGGTGCGATC | CTTGTAGTAG | CATCTACTGA | CGGACCAATG | CCACAAACTC | GTGAGCACAT | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| CCTTCTTTCA | CGTCAGGTTG | GTGTTAAACA | CCTTATCGTC | TTCATGAACA | AAGTTGACTT | 120 |
| GGTTGACGAT | GAAGAATTGC | TTGAATTGGT | TGAAATGGAA | ATCCGTGACC | TTCTTTCAGA | 180 |
| ATACGATTTC | CCAGGTGATG | ACATTCCAGT | TATCCAAGGT | TCAGCTCTTA | AAGCTCTTGA | 240 |
| AGGTGATTCT | AAATACGAAG | ACATCATCAT | GGACTTGATG | AACACTGTTG | ACGAATACAT | 300 |
| CCCAGAACCA | GAACGTGACA | CTGACAAACC | ATTGTTGCTT | CCAGTCGAAG | ACGTATTCTC | 360 |
| AATCACTGGT | CGTGGTACTG | TTGCTTCAGG | ACGTATCGAC | CGTGGTGTTG | TTCGTGTCAA | 420 |
| TGACGAAGTT | GAAATCGTTG | GTCTTAAAGA | AGACATCCAA | AAAGCAGTTG | TTACTGGTGT | 480 |
| TGAAATGTTC | CGTAAACAAC | TTGACGRAGG | TATTGCCGGA | GATAACGTCG | GTGTTCTTCT | 540 |
| TCGTGGTATC | CAACGTGATG | AAATCGAACG | TGGTCAAGTA | TTGGCTGCAC | CTGGTTCAAT | 600 |
| CAACCCACAC | ACTAAATTCA | AAGGTGAAGT | TTACATCCTT | TCTAAAGAAG | AAGGTGGACG | 660 |
| TCACACTCCA | TTCTTCAACA | ACTACCGTCC | ACAGTTCTAC | TTCCGTACAA | CTGACGTAAC | 720 |
| AGGTTCAATC | GAACTTCCTG | CAGGTACTGA | AATGGTTATG | CCTGGTGATA | ACGTGACTAT | 780 |
| CGACGTTGAG | TTGATCCACC | CAATCGCCGT | TGAACAA    |            |            | 817 |

- (2) INFORMATION FOR SEQ ID NO: 147:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 897 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Agrobacterium tumefaciens
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:
- AACATGATCA CCGGTGCTGC CGAGATGGAC GGCGCGATCC TGGTTTGCTC GGCTGCCGAC 60
  GGCCCGATGC CACAGACCCG CGAGCACATC CTGCTTGCCC GTCAGGTGGG CGTTCCGGCC 120

| ATCGTCGTGT | TCCTCAACAA | GGTCGACCAG | GTTGACGACG | CCGAGCTTCT | CGAGCTCGTC | 180 |
|------------|------------|------------|------------|------------|------------|-----|
| GAGCTTGAAG | TTCGCGAACT | TCTGTCGTCC | TACGACTTCC | CGGGCGACGA | TATCCCGATC | 240 |
| ATCAAGGGTT | CGGCACTTGC | TGCTCTTGAA | GATTCTGACA | AGAAGATCGG | TGAAGACGCG | 300 |
| ATCCGCGAGC | TGATGGCTGC | TGTCGACGCC | TACATCCCGA | CGCCTGAGCG | TCCGATCGAC | 360 |
| CAGCCGTTCC | TGATGCCGAT | CGAAGACGTG | TTCTCGATCT | CGGGTCGTGG | TACGGTTGTG | 420 |
| ACGGGTCGCG | TTGAGCGCGG | TATCGTCAAG | GTTGGTGAAG | AAGTCGAAAT | CGTCGGCATC | 480 |
| CGTCCGACCT | CGAAGACGAC | TGTTACCGGC | GTTGAAATGT | TCCGCAAGCT | GCTCGACCAG | 540 |
| GGCCAGGCCG | GCGACAACAT | CGGTGCACTC | GTTCGCGGCG | TTACCCGTGA | CGGCGTCGAG | 600 |
| CGTGGTCAGA | TCCTGTGCAA | GCCGGGTTCG | GTCAAGCCGC | ACAAGAAGTT | CATGGCAGAA | 660 |
| GCCTACATCC | TGACGAAGGA | AGAAGGCGGC | CGTCATACGC | CGTTCTTCAC | GAACTACCGT | 720 |
| CCGCAGTTCT | ACTTCCGTAC | GACTGACGTT | ACCGGTATCG | TTTCGCTTCC | TGAAGGCACG | 780 |
| GAAATGGTTA | TGCCTGGCGA | CAACGTCACT | GTTGAAGTCG | AGCTGATCGT | TCCGATCGCG | 840 |
| ATGGAAGAAA | AGCTGCGCTT | CGCTATCCGC | GAAGGCGGCC | GTACCGTCGG | CGCCGGC    | 897 |

- (2) INFORMATION FOR SEQ ID NO: 148:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 885 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

2.0

- (A) ORGANISM: Bacillus subtilis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

| 60  | TGCTGATGGC | TAGTATCTGC | GCTATCCTTG | AATGGACGGA | GTGCTGCGCA | ATGATCACTG |
|-----|------------|------------|------------|------------|------------|------------|
| 120 | ACCATACATC | ACGTTGGTGT | CTTTCTAAAA | GCACATCCTT | AAACTCGTGA | CCAATGCCAC |
| 180 | ACTAGTTGAA | AGCTTCTTGA | GACGACGAAG | CGACATGGTA | TAAACAAATG | GTTGTATTCT |
| 240 | ACCAGTTGTT | GTGATGATGT | GACTTCCCTG | TAGCGAATAC | GCGATCTTCT | ATGGAAGTTC |
| 300 | AATCTTCGAA | GGGAAGCTAA | GACGCTGAGT | TCTTGAAGGA | CTCTTAAAGC | AAAGGTTCTG |
| 360 | AAAACCATTC | GCGACACTGA | ACTCCAGAAC | GTACATCCCA | CGGTTGATGA | CTTATGGATG |
| 420 | TACTGGCCGT | GTACAGTTGC | ACTGGTCGTG | ATTCTCAATC | TTGAGGACGT | ATGATGCCAG |
| 480 | TCAAGAAGAG | TCATCGGTCT | GAAGTTGAAA | AGTCGGTGAC | GACAAGTTAA | GTAGAACGCG |

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AACAAGAAA CAACTGTTAC AGGTGTTGAA ATGTTCCGTA AGCTTCTTGA TTACGCTGAA 540
GCTGGTGACA ACATTGGTGC CCTTCTTCGC GGTGTATCTC GTGAAGAAAT CCAACGTGGT 600
CAAGTACTTG CTAAACCAGG TACAATCACT CCACACAGCA AATTCAAAGC TGAAGTTTAC 660
GTTCTTTCTA AAGAAGAGGG TGGACGTCAT ACTCCATTCT TCTCTAACTA CCGTCCTCAG 720
TTCTACTTCC GTACAACTGA CGTAACTGGT ATCATCCATC TTCCAGAAGG CGTAGAAATG 780
GTTATGCCTG GAGATAACAC TGAAATGAAC GTTGAACTTA TTTCTACAAT CGCTATCGAA 840
GAAGGAACTC GTTTCTCTAT TCGTGAAGGC GGACGTACTG TTGGT 885

- (2) INFORMATION FOR SEQ ID NO: 149:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 882 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Bacteroides fragilis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

ATGGTTACTG GTGCTGCTCA GATGGACGGT GCTATCATTG TAGTTGCTGC TACTGATGGT 60 CCGATGCCTC AGACTCGTGA GCACATCCTT TTGGCTCGTC AGGTAAACGT TCCGAAGCTG 120 GTTGTATTCA TGAACAAGTG CGATATGGTT GAAGATGCTG AGATGTTGGA ACTTGTTGAA 180 ATGGAAATGA GAGAATTGCT TTCATTCTAT GATTTCGACG GTGACAATAC TCCGATCATT CAGGGTTCTG CTCTTGGTGC ATTGAACGGC GTAGAAAAAT GGGAAGACAA AGTAATGGAA 300 CTGATGGAAG CTGTTGATAC TTGGATTCCA CTGCCTCCGC GCGATGTTGA TAAACCTTTC 360 TTGATGCCGG TAGAAGACGT GTTCTCTATC ACAGGTCGTG GTACTGTAGC TACAGGTCGT 420 ATCGAAACTG GTGTTATCCA TGTAGGTGAT GAAATCGAAA TCCTCGGTTT GGGTGAAGAT 480 AAGAAATCAG TTGTAACAGG TGTTGAAATG TTCCGCAAAC TTCTGGATCA GGGTGAAGCT 540 GGTGACAACG TAGGTCTGTT GCTTCGTGGT GTTGACAAGA ACGAAATCAA ACGTGGTATG 600 GTTCTTTGTA AACCGGGTCA GATTAAACCT CACTCTAAAT TCAAAGCAGA GGTTTATATC 660 CTGAAGAAG AAGAAGGTGG TCGTCACACT CCATTCCATA ACAAATATCG TCCTCAGTTC 720 TACCTGCGTA CTATGGACTG TACAGGTGAA ATCACTCTTC CGGAAGGAAC TGAAATGGTA 780 ATGCCGGGTG ATAACGTAAC TATCACTGTA GAGTTGATCT ATCCGGTTGC ACTGAACATC 840

# GGTCTTCGTT TCGCTATCCG CGAAGGTGGA CGTACAGTAG GT 882

- (2) INFORMATION FOR SEQ ID NO: 150:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 888 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Borrelia burgdorferi
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

AATATGATTA CAGGAGCAGC TCAAATGGAT GCAGCGATAC TTTTAGTTGC TGCTGATAGT 60 GGTGCTGAGC CTCAAACAAA AGAGCATTTG CTTCTTGCTC AAAGAATGGG AATAAAGAAA 120 ATAATAGTTT TTTTAAATAA ATTGGACTTA GCAGATCCTG AACTTGTTGA GCTTGTTGAA 180 GTTGAAGTTT TAGAACTTGT TGAAAAATAT GGCTTTTCAG CTGATACTCC AATAATCAAA 240 GGTTCAGCTT TTGGGGCTAT GTCAAATCCA GAAGATCCTG AATCTACAAA ATGCGTTAAA 300 GAACTTCTTG AATCTATGGA TAATTATTTT GATCTTCCAG AAAGAGATAT TGACAAGCCA 360 TTTTTGCTTG CTGTTGAAGA TGTATTTTCT ATTTCAGGAA GAGGCACTGT TGCTACTGGG 420 CGTATTGAAA GAGGTATTAT TAAAGTTGGT CAAGAAGTTG AAATAGTTGG AATTAAAGAA 480 ACCAGAAAAA CTACTGTTAC TGGTGTTGAA ATGTTCCAGA AAATTCTTGA GCAAGGTCAA 540 GCAGGGGATA ATGTTGGTCT TCTTTTGAGA GGCGTTGATA AAAAAGACAT TGAGAGGGGG CAAGTTTTGT CAGCTCCAGG TACAATTACT CCACACAAGA AATTTAAAGC TTCAATTTAT 660 TGTTTGACTA AAGAAGAAGG CGGTAGGCAC AAGCCATTTT TCCCAGGGTA TAGACCACAG 720 TTCTTTTTTA GAACAACCGA TGTTACTGGA GTTGTTGCTT TAGAGGGCAA AGAAATGGTT 780 ATGCCTGGTG ATAATGTTGA TATTATTGTT GAGCTGATCT CTTCAATAGC TATGGATAAG 840 AATGTAGAAT TTGCTGTTCG AGAAGGTGGA AGAACCGTTG CTTCAGGA 888

- (2) INFORMATION FOR SEQ ID NO: 151:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 894 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

| (iv) | ORIGINAL | SOURCE: |
|------|----------|---------|
|------|----------|---------|

(A) ORGANISM: Brevibacterium linens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

| AACATGATCA | CCGGTGCCGC | TCAGATGGAC | GGTGCGATCC | TCGTCGTCGC | CGCTACCGAC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| GGACCGATGC | CCCAGACCCG | TGAGCACGTG | CTGCTCGCGC | GTCAGGTCGG | CGTTCCCTAC | 120 |
| ATCGTCGTGG | CTCTGAACAA | GTCCGACATG | GTCGATGACG | AGGAGCTCCT | CGAGCTCGTC | 180 |
| GAATTCGAGG | TCCGCGACCT | GCTCTCGAGC | CAGGACTTCG | ACGGAGACAA | CGCTCCGGTC | 240 |
| ATTCCGGTGT | CCGCTCTCAA | GGCGCTGGAA | GGCGACGAGA | AGTGGGTCAA | GAGCGTTCAG | 300 |
| GATCTCATGG | CTGCCGTCGA | TGACAACGTT | CCGGAGCCGG | AGCGCGATGT | CGACAAGCCG | 360 |
| TTCCTCATGC | CCGTCGAGGA | CGTCTTCACG | ATCACCGGTC | GTGGAACCGT | CGTCACCGGT | 420 |
| CGTGTCGAGC | GCGGCGTGCT | CCTGCCTAAC | GACGAAATCG | AAATCGTCGG | CATCAAGGAG | 480 |
| AAGTCGTCCA | AGACGACTGT | CACCGCTATC | GAGATGTTCC | GCAAGACCCT | GCCGGATGCC | 540 |
| CGTGCAGGTG | AGAACGTCGG | TCTGCTCCTC | CGCGGCACCA | AGCGCGAGGA | TGTTGAGCGC | 600 |
| GGTCAGGTCA | TCGTGAAGCC | GGGTTCGATC | ACCCCGCACA | CCAAGTTCGA | GGCTCAGGTC | 660 |
| TACATCCTGA | GCAAGGACGA | GGGCGGACGT | CACAACCCGT | TCTACTCGAA | CTACCGTCCG | 720 |
| CAGTTCTACT | TCCGGACCAC | GGACGTCACC | GGTGTCATCA | CGCTGCCCGA | GGGCACCGAG | 780 |
| ATGGTCATGC | CCGGCGACAA | CACCGATATG | TCGGTCGAGC | TCATCCAGCC | GATCGCTATG | 840 |
| GAGGACCGCC | TCCGCTTCGC | AATCCGCGAA | GGTGGCCGCA | CCGTCGGCGC | CGGT       | 894 |

## (2) INFORMATION FOR SEQ ID NO: 152:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 888 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Burkholderia cepacia
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

ATGATCACGG GCGCAGCGCA GATGGACGGC GCGATCCTGG TTTGCTCGGC AGCAGACGGC 60
CCGATGCCGC AAACGCGTGA GCACATCCTG CTGGCGCGTC AGGTTGGTGT TCCGTACATC 120
ATCGTGTTCC TGAACAAGTG CGACAGTGTG GACGACGCTG AACTGCTCGA GCTGGTCGAG 180

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| ATGGAAGTTC | GCGAACTCCT | GTCGAAGTAC | GACTTCCCGG | GCGACGACAC | GCCGATCGTG | 240 |
|------------|------------|------------|------------|------------|------------|-----|
| AAGGGTTCGG | CCAAGCTGGC | GCTGGAAGGC | GACACGGGCG | AGCTGGGCGA | AGTGGCGATC | 300 |
| ATGAGCCTGG | CAGACGCGCT | GGACACGTAC | ATCCCGACGC | CGGAGCGTGC | AGTTGACGGC | 360 |
| GCGTTCCTGA | TGCCGGTGGA | AGACGTGTTC | TCGATCTCGG | GCCGTGGTAC | GGTGGTGACG | 420 |
| GGTCGTGTCG | AGCGCGGCAT | CGTGAAGGTC | GGCGAAGAAA | TCGAAATCGT | CGGTATCAAG | 480 |
| CCGACGGTGA | AGACGACCTG | CACGGGCGTT | GAAATGTTCC | GCAAGCTGCT | GGACCAAGGT | 540 |
| CAGGCAGGCG | ACAACGTCGG | TATCCTGCTG | CGCGGCACGA | AGCGTGAAGA | CGTGGAGCGT | 600 |
| GGCCAGGTTC | TGGCGAAGCC | GGGTTCGATC | ACGCCGCACA | CGCACTTCAC | GGCTGAAGTG | 660 |
| TACGTGCTGA | GCAAGGACGA | AGGCGGCCGT | CACACGCCGT | TCTTCAACAA | CTACCGTCCG | 720 |
| CAGTTCTACT | TCCGTACGAC | GGACGTGACG | GGCTCGATCG | AGCTGCCGAA | GGACAAGGAA | 780 |
| ATGGTGATGC | CGGGCGACAA | CGTGTCGATC | ACGGTGAAGC | TGATTGCTCC | GATCGCGATG | 840 |
| GAAGAAGGTC | TGCGCTTCGC | AATCCGTGAA | GGCGGCCGTA | CGGTCGGC   |            | 888 |

- (2) INFORMATION FOR SEQ ID NO: 153:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Chlamydia trachomatis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

60 AACATGATCA CCGGTGCGGC TCAAATGGAC GGGGCTATTC TAGTAGTTTC TGCAACAGAC GGAGCTATGC CTCAAACTAA AGAGCATATT CTTTTGGCAA GACAAGTTGG GGTTCCTTAC 120 ATCGTTGTTT TTCTCAATAA AATTGACATG ATTTCCGAAG AAGACGCTGA ATTGGTCGAC 180 TTGGTTGAGA TGGAGTTGGC TGAGCTTCTT GAAGAGAAAG GATACAAAGG GTGTCCAATC 240 300 ATCAGAGGTT CTGCTCTGAA AGCTTTGGAA GGAGATGCTG CATACATAGA GAAAGTTCGA GAGCTAATGC AAGCCGTCGA TGATAATATC CCTACTCCAG AAAGAGAAAT TGACAAGCCT 360 TTCTTAATGC CTATTGAGGA CGTGTTCTCT ATCTCCGGAC GAGGAACTGT AGTAACTGGA 420 CGTATTGAGC GTGGAATTGT TAAAGTTTCC GATAAAGTTC AGTTGGTCGG TCTTAGAGAT 480 ACTAAAGAAA CGATTGTTAC TGGGGTTGAA ATGTTCAGAA AAGAACTCCC AGAAGGTCGT 540

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| GCAGGAGAGA | ACGTTGGATT | GCTCCTCAGA | GGTATTGGTA | AGAACGATGT | GGAAAGAGGA | 600 |
|------------|------------|------------|------------|------------|------------|-----|
| ATGGTTGTTT | GCTTGCCAAA | CAGTGTTAAA | CCTCATACAC | AGTTTAAGTG | TGCTGTTTAC | 660 |
| GTTCTGCAAA | AAGAAGAAGG | TGGACGACAT | AAGCCTTTCT | TCACAGGATA | TAGACCTCAA | 720 |
| TTCTTCTTCC | GTACAACAGA | CGTTACAGGT | GTGGTAACTC | TGCCTGAGGG | AGTTGAGATG | 780 |
| GTCATGCCTG | GGGATAACGT | TGAGTTTGAA | GTGCAATTGA | TTAGCCCTGT | GGCTTTAGAA | 840 |
| GAAGGTATGA | GATTTGCGAT | TCGTGAAGGT | GGTCGTACAA | TCGGTGCTGG | A .        | 891 |

## (2) INFORMATION FOR SEQ ID NO: 154:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Escherichia coli
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

| AACATGATCA | CCGGTGCTGC | GCAGATGGAC | GGCGCGATCC | TGGTAGTTGC | TGCGACTGAC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| GGCCCGATGC | CGCAGACTCG | TGAGCACATC | CTGCTGGGTC | GTCAGGTAGG | CGTTCCGTAC | 120 |
| ATCATCGTGT | TCCTGAACAA | ATGCGACATG | GTTGATGACG | AAGAGCTGCT | GGAACTGGTT | 180 |
| GAAATGGAAG | TTCGTGAACT | TCTGTCTCAG | TACGACTTCC | CGGGCGACGA | CACTCCGATC | 240 |
| GTTCGTGGTT | CTGCTCTGAA | AGCGCTGGAA | GGCGACGCAG | AGTGGGAAGC | GAAAATCCTG | 300 |
| GAACTGGCTG | GCTTCCTGGA | TTCTTACATT | CCGGAACCAG | AGCGTGCGAT | TGACAAGCCG | 360 |
| TTCCTGCTGC | CGATCGAAGA | CGTATTCTCC | ATCTCCGGTC | GTGGTACCGT | TGTTACCGGT | 420 |
| CGTGTAGAAC | GCGGTATCAT | CAAAGTTGGT | GAAGAAGTTG | AAATCGTTGG | TATCAAAGAG | 480 |
| ACTCAGAAGT | CTACCTGTAC | TGGCGTTGAA | ATGTTCCGCA | AACTGCTGGA | CGAAGGCCGT | 540 |
| GCTGGTGAGA | ACGTAGGTGT | TCTGCTGCGT | GGTATCAAAC | GTGAAGAAAT | CGAACGTGGT | 600 |
| CAGGTACTGG | CTAAGCCGGG | CACCATCAAG | CCGCACACCA | AGTTCGAATC | TGAAGTGTAC | 660 |
| ATTCTGTCCA | AAGATGAAGG | CGGCCGTCAT | ACTCCGTTCT | TCAAAGGCTA | CCGTCCGCAG | 720 |
| TTCTACTTCC | GTACTACTGA | CGTGACTGGT | ACCATCGAAC | TGCCGGAAGG | CGTAGAGATG | 780 |
| GTAATGCCGG | GCGACAACAT | CAAAATGGTT | GTTACCCTGA | TCCACCCGAT | CGCGATGGAC | 840 |
| GACGGTCTGC | GTTTCGCAAT | CCGTGAAGGC | GGCCGTACCG | TTGGCGCGGG | C          | 891 |

| (2) | INFORMATION | FOR | SEO | ID | NO: | 155: |
|-----|-------------|-----|-----|----|-----|------|
|-----|-------------|-----|-----|----|-----|------|

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Fibrobacter succinogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

| AACATGGTGA CTGGTGCTGC | TCAGATGGAC | GGCGCTATCC | TCGTTGTTGC | CGCTACTGAC | 60  |
|-----------------------|------------|------------|------------|------------|-----|
| GGTCCGATGC CGCAGACTCG | CGAACACATC | CTTCTCGCTC | ACCAGGTTGG | CGTGCCGAAG | 120 |
| ATCGTCGTGT TCATGAACAA | GTGCGACATG | GTTGACGATG | CTGAAATTCT | CGACCTCGTC | 180 |
| GAAATGGAAG TTCGCGAACT | CCTCTCCAAG | TATGACTTCG | ACGGTGACAA | CÁCCCCGATC | 240 |
| ATCCGTGGTT CCGCTCTCAA | GGCCCTCGAA | GGCGATCCGG | AATACCAGGA | CAAGGTCATG | 300 |
| GAACTCATGA ACGCTTGCGA | CGAATACATC | CCGCTCCCGC | AGCGCGATAC | CGACAAGCCG | 360 |
| TTCCTCATGC CGATCGAAGA | CGTGTTCACG | ATTACTGGCC | GCGGCACTGT | CGCTACTGGC | 420 |
| CGTATCGAAC GCGGTGTCGT | TCGCTTGAAC | GACAAGGTTG | AACGTATCGG | TCTCGGTGAA | 480 |
| ACCACCGAAT ACGTCATCAC | CGGTGTTGAA | ATGTTCCGTA | AGCTCCTCGA | CGACGCTCAG | 540 |
| GCAGGTGACA ACGTTGGTCT | CCTCCTCCGT | GGTGCTGAAA | AGAAGGACAT | CGTCCGTGGC | 600 |
| ATGGTTCTCG CAGCTCCGAA | GTCTGTCACT | CCGCACACCG | AATTTAAGGC | TGAAATCTAC | 660 |
| GTTCTCACGA AGGACGAAGG | TGGCCGTCAC | ACGCCGTTCA | TGAATGGCTA | CCGTCCGCAG | 720 |
| TTCTACTTCC GCACCACCGA | CGTTACTGGT | ACGATCCAGC | TCCCGGAAGG | TGTCGAAATG | 780 |
| GTTACTCCGG GTGACACGGT | CACGATCCAC | GTGAACCTCA | TCGCTCCGAT | CGCTATGGAA | 840 |
| AAGCAGCTCC GCTTCGCTAT | CCGTGAAGGT | GGACGTACTG | TTGGTGCTGG | С          | 891 |

- (2) INFORMATION FOR SEQ ID NO: 156:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 894 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

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## (A) ORGANISM: Flavobacterium ferrugineum

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:

| AACATGATCA | CCGGTGCTGC | CCAGATGGAC | GGTGCTATCT | TAGTTGTGGC | TGCATCAGAC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| GGTCCTATGC | CTCAAACAAA | AGAACACATC | CTGCTTGCTG | CCCAGGTAGG | TGTACCTAAA | 120 |
| ATGGTTGTGT | TTCTGAATAA | AGTTGACCTC | GTTGACGACG | AAGAGCTCCT | GGAGCTGGTT | 180 |
| GAGATCGAGG | TTCGCGAAGA | ACTGACTAAA | CGCGGTTTCG | ACGGCGACAA | CACTCCAATC | 240 |
| ATCAAAGGTT | CCGCTACAGG | CGCCCTCGCT | ĢĢTGAAGAAA | AGTGGGTTAA | AGAAATTGAA | 300 |
| AACCTGATGG | ACGCTGTTGA | CAGCTACATC | CCACTGCCTC | CTCGTCCGGT | TGATCTGCCG | 360 |
| TTCCTGATGA | GCGTAGAGGA | CGTATTCTCT | ATCACTGGTC | GTGGTACTGT | TGCTACCGGT | 420 |
| CGTATCGAGC | GTGGCCGTAT | CAAAGTTGGT | GAGCCTGTTG | AGATCGTAGG | TCTGCAGGAG | 480 |
| TCTCCCCTGA | ACTCTACCGT | TACAGGTGTT | GAGATGTTCC | GCAAACTCCT | CGACGAAGGT | 540 |
| GAAGCTGGTG | ATAACGCCGG | TCTCCTCCTC | CGTGGTGTTG | AAAAAACACA | GATCCGTCGC | 600 |
| GGTATGGTAA | TCGTTAAACC | CGGTTCCATC | ACTCCGCACA | CGGACTTCAA | AGGCGAAGTT | 660 |
| TACGTACTGA | GCAAAGACGA | AGGTGGCCGT | CACACTCCAT | TCTTCAACAA | ATACCGTCCT | 720 |
| CAATTCTACT | TCCGTACAAC | TGACGTTACA | GGTGAAGTAG | AACTGAACGC | AGGAACAGAA | 780 |
| ATGGTTATGC | CTGGTGATAA | CACCAACCTG | ACCGTTAAAC | TGATCCAACC | GATCGCTATG | 840 |
| GAAAAAGGTC | TGAAATTCGC | GATCCGCGAA | GGTGGCCGTA | CCGTAGGTGC | AGGA       | 894 |

## (2) INFORMATION FOR SEQ ID NO: 157:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Haemophilus influenzae
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:

AATATGATTA CTGGTGCGGC ACAAATGGAT GGTGCTATTT TAGTAGTAGC AGCAACAGAT 60

GGTCCTATGC CACAAACTCG TGAACACATC TTATTAGGTC GCCAAGTAGG TGTTCCATAC 120

ATCATCGTAT TCTTAAACAA ATGCGACATG GTAGATGACG AAGAGTTATT AGAATTAGTC 180

GAAATGGAAG TTCGTGAACT TCTATCTCAA TATGACTTCC CAGGTGACGA TACACCAATC 240

| GTAC  | STGGTT | CAGCATTACA | AGCGTTAAAC | GGCGTAGCAG | AATGGGAAGA | AAAAATCCTT | 300 |
|-------|--------|------------|------------|------------|------------|------------|-----|
| GAGT  | TAGCAA | ACCACTTAGA | TACTTACATC | CCAGAACCAG | AACGTGCGAT | TGACCAACCG | 360 |
| TTCCI | TCTTC  | CAATCGAAGA | TGTGTTCTCA | ATCTCAGGTC | GTGGTACTGT | AGTAACAGGT | 420 |
| CGTGT | TAGAAC | GAGGTATTAT | CCGTACAGGT | GATGAAGTAG | AAATCGTCGG | TATCAAAGAT | 480 |
| ACAGO | GAAAA  | CTACTGTAAC | GGGTGTTGAA | ATGTTCCGTA | AATTACTTGA | CGAAGGTCGT | 540 |
| GCAGG | ETGAAA | ACATCGGTGC | ATTATTACGT | GGTACCAAAC | GTGAAGAAAT | CGAACGTGGT | 600 |
| CAAGT | TATTAG | CGAAACCAGG | TTCAATCACA | CCACACACTG | ACTTCGAATC | AGAAGTGTAC | 660 |
| GTATI | TATCAA | AAGATGAAGG | TGGTCGTCAT | ACTCCATTCT | TCAAAGGTTA | CCGTCCACAA | 720 |
| TTCTA | ATTTCC | GTACAACAGA | CGTGACTGGT | ACAATCGAAT | TACCAGAAGG | CGTGGAAATG | 780 |
| GTAAT | GCCAG  | GCGATAACAT | CAAGATGACA | GTAAGCTTAA | TCCACCCAAT | TGCGATGGAT | 840 |
| CAAGO | STTTAC | GTTTCGCAAT | CCGTGAAGGT | GGCCGTACAG | TAGGTGCAGG | С          | 891 |
|       |        |            |            |            |            |            |     |

- (2) INFORMATION FOR SEQ ID NO: 158:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 906 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Helicobacter pylori
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:

AACATGATCA CCGGTGCGGC GCAAATGGAC GGAGCGATTT TGGTTGTTTC TGCAGCTGAT 60 GGCCCTATGC CTCAAACTAG GGAGCATATC TTATTGTCTC GTCAAGTAGG CGTGCCTCAC 120 ATCGTTGTTT TCTTAAACAA ACAAGACATG GTAGATGACC AAGAATTGTT AGAACTTGTA 180 GAAATGGAAG TGCGCGAATT GTTGAGCGCG TATGAATTTC CTGGCGATGA CACTCCTATC 240 GTAGCGGGTT CAGCTTTAAG AGCTTTAGAA GAAGCAAAGG CTGGTAATGT GGGTGAATGG 300 GGTGAAAAAG TGCTTAAACT TATGGCTGAA GTGGATGCCT ATATCCCTAC TCCAGAAAGA 360 GACACTGAAA AAACTTTCTT GATGCCGGTT GAAGATGTGT TCTCTATTGC GGGTAGAGGG 420 ACTGTGGTTA CAGGTAGGAT TGAAAGAGGC GTGGTGAAAG TAGGCGATGA AGTGGAAATC 480 GTTGGTATCA GACCTACACA AAAAACGACT GTAACCGGTG TAGAAATGTT TAGGAAAGAG 540 TTGGAAAAAG GTGAAGCCGG CGATAATGTG GGCGTGCTTT TGAGAGGAAC TAAAAAAGAA 600 WO 98/20157 PCT/CA97/00829 -

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| GAAGTGGAAC | GCGGTATGGT | TCTATGCAAA | CCAGGTTCTA | TCACTCCGCA | CAAGAAATTT | 660 |
|------------|------------|------------|------------|------------|------------|-----|
| GAGGGAGAAA | TTTATGTCCT | TTCTAAAGAA | GAAGGCGGGA | GACACACTCC | ATTCTTCACC | 720 |
| AATTACCGCC | CGCAATTCTA | TGTGCGCACA | ACTGATGTGA | CTGGCTCTAT | CACCCTTCCT | 780 |
| GAAGGCGTAG | AAATGGTTAT | GCCTGGCGAT | AATGTGAAAA | TCACTGTAGA | GTTGATTAGC | 840 |
| CCTGTTGCGT | TAGAGTTGGG | AACTAAATTT | GCGATTCGTG | AAGGCGGTAG | GACCGTTGGT | 900 |
| GCTGGT     |            |            |            |            |            | 906 |

#### (2) INFORMATION FOR SEQ ID NO: 159:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Micrococcus luteus
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:

| AACATGATCA | CCGGCGCCGC | TCAGATGGAC | GGCGCGATCC | TCGTGGTCGC | CGCTACCGAC | 60  |
|------------|------------|------------|------------|------------|------------|-----|
| GGCCCGATGG | CCCAGACCCG | TGAGCACGTG | CTCCTGGCCC | GCCAGGTCGG | CGTGCCGGCC | 120 |
| CTGCTCGTGG | CCCTGAACAA | GTCGGACATG | GTGGAGGACG | AGGAGCTCCT | CGAGCGTGTC | 180 |
| GAGATGGAGG | TCCGGCAGCT | GCTGTCCTCC | AGGAGCTTCG | ACGTCGACGA | GGCCCCGGTC | 240 |
| ATCCGCACCT | CCGCTCTGAA | GGCCCTCGAG | GGCGACCCCC | AGTGGGTCAA | GTCCGTCGAG | 300 |
| GACCTCATGG | ATGCCGTGGA | CGAGTACATC | CCGGACCCGG | TGCGCGACAA | GGACAAGCCG | 360 |
| TTCCTGATGC | CGATCGAGGA | CGTCTTCACG | ATCACCGGCC | GTGGCACCGT | GGTGACCGGT | 420 |
| CGCGCCGAGC | GCGGCACCCT | GAAGATCAAC | TCCGAGGTCG | AGATCGTCGG | CATCCGCGAC | 480 |
| GTGCAGAAGA | CCACTGTCAC | CGGCATCGAG | ATGTTCCACA | AGCAGCTCGA | CGAGGCCTGG | 540 |
| GCCGGCGAGA | ACTGCGGTCT | GCTCGTGCGC | GGTCTGAAGC | GCGACGACGT | CGAGCGCGGC | 600 |
| CAGGTGCTGG | TGGAGCCGGG | CTCCATCACC | CCGCACACCA | ACTTCGAGGC | GAACGTCTAC | 660 |
| ATCCTGTCCA | AGGACGAGGG | TGGGCGTCAC | ACCCCGTTCT | ACTCGAACTA | CCGCGCGCAG | 720 |
| TTCTACTTCC | GCACCACCGA | CGTCACCGGC | GTCATCACGC | TGCCCGAGGG | CACCGAGATG | 780 |
| GTCATGCCCG | GCGACACCAC | CGAGATGTCG | GTCGAGCTCA | TCCAGCCGAT | CGCCATGGAG | 840 |
| GAGGGCCTCG | GCTTCGCCAT | CCGCGAGGGT | GGCCGCACCG | TGGGCTCCGG | С          | 891 |

#### (2) INFORMATION FOR SEQ ID NO: 160:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Mycobacterium tuberculosis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:

AACATGATCA CCGGCGCCGC GCAGATGGAC GGTGCGATCC TGGTGGTCGC CGCCACCGAC 60 GGCCCGATGC CCCAGACCCG CGAGCACGTT CTGCTGGCGC GTCAAGTGGG TGTGCCCTAC 120 ATCCTGGTAG CGCTGAACAA GGCCGACGCA GTGGACGACG AGGAGCTGCT CGAACTCGTC 180 GAGATGGAGG TCCGCGAGCT GCTGGCTGCC CAGGAATTCG ACGAGGACGC CCCGGTTGTG 240 CGGGTCTCGG CGCTCAAGGC GCTCGAGGGT GACGCGAAGT GGGTTGCCTC TGTCGAGGAA 300 CTGATGAACG CGGTCGACGA GTCGATTCCG GACCCGGTCC GCGAGACCGA CAAGCCGTTC 360 CTGATGCCGG TCGAGGACGT CTTCACCATT ACCGGCCGCG GAACCGTGGT CACCGGACGT 420 GTGGAGCGCG GCGTGATCAA CGTGAACGAG GAAGTTGAGA TCGTCGGCAT TCGCCCATCG 480 540 ACCACCAAGA CCACCGTCAC CGGTGTGGAG ATGTTCCGCA AGCTGCTCGA CCAGGGCCAG GCGGGCGACA ACGTTGGTTT GCTGCTGCGG GGCGTCAAGC GCGAGGACGT CGAGCGTGGC 600 CAGGTTGTCA CCAAGCCCGG CACCACCACG CCGCACACCG AGTTCGAAGG CCAGGTCTAC 660 ATCCTGTCCA AGGACGAGGG CGGCCGCAC ACGCCGTTCT TCAACAACTA CCGTCCGCAG 720 TTCTACTTCC GCACCACCGA CGTGACCGGT GTGGTGACAC TGCCGGAGGG CACCGAGATG 780 GTGATGCCCG GTGACAACAC CAACATCTCG GTGAAGTTGA TCCAGCCCGT CGCCATGGAC 840 891 GAAGGTCTGC GTTTCGCGAT CCGCGAGGGT GGCCGCACCG TGGGCGCCGG C

- (2) INFORMATION FOR SEQ ID NO: 161:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs
      (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

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| (A) | ORGANISM: | Mycoplasma | genitalium |
|-----|-----------|------------|------------|
|-----|-----------|------------|------------|

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161: AATATGATCA CAGGTGCTGC ACAAATGGAT GGAGCTATTC TAGTTGTTTC AGCAACTGAT 60 AGTGTGATGC CCCAAACCCG CGAGCACATC TTACTTGCCC GCCAAGTAGG GGTTCCTAAA 120 ATGGTAGTTT TTCTAAACAA GTGTGATATT GCTAGTGATG AAGAGGTACA AGAACTTGTT 180 GCTGAAGAAG TACGTGATCT GTTAACTTCC TATGGTTTTG ATGGTAAGAA CACTCCTATT 240 . ATTTATGGCT CAGCTTTAAA AGCATTGGAA GGTGATCCAA AGTGGGAGGC TAAGATCCAT 300 360 GATTTGATTA AAGCAGTTGA TGAATGGATT CCAACTCCTA CACGTGAAGT AGATAAACCT TTCTTATTAG CAATTGAAGA TACGATGACC ATTACTGGTA GAGGTACAGT TGTTACAGGA 420 AGAGTTGAAA GAGGTGAACT CAAAGTAGGT CAAGAAGTTG AAATTGTTGG TTTAAAACCA 480 ATTAGAAAAG CAGTTGTTAC TGGAATTGAA ATGTTCAAAA AGGAACTTGA TTCAGCAATG 540 600 GCTGGTGACA ATGCTGGGGT ATTATTACGT GGTGTTGAAC GTAAAGAAGT TGAAAGAGGT CAAGTTTTAG CAAAACCAGG CTCTATTAAA CCGCACAAGA AATTTAAAGC TGAGATCTAT 660 720 GCTTTAAAGA AAGAAGAAGG TGGTAGACAC ACTGGTTTTT TAAACGGTTA CCGTCCTCAA 780 TTCTATTTCC GTACCACTGA TGTAACTGGT TCTATTGCTT TAGCTGAAAA TACTGAAATG GTTCTACCTG GTGATAATGC TTCTATTACT GTTGAGTTAA TTGCTCCTAT CGCTTGTGAA 840

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#### (2) INFORMATION FOR SEO ID NO: 162:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Neisseria gonorrheae

AAAGGTAGTA AGTTCTCAAT TCGTGAAGGT GGTAGAACTG TAGGGGCAGG C

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

AACATGATTA CCGGCGCCGC ACAAATGGAC GGTGCAATCC TGGTATGTTC TGCTGCCGAC 60
GGCCCTATGC CGCAAACCCG CGAACACATC CTGCTGGCCC GTCAAGTAGG CGTACCTTAC 120
ATCATCGTGT TCATGAACAA ATGCGACATG GTCGACGATG CCGAGCTGTT CCAACTGGTT 180
GAAATGGAAA TCCGCGACCT GCTGTCCAGC TACGACTTCC CCGGCGACGA CTGCCCGATC 240

| GTACAAGGTT | CCGCACTGAA | AGCCTTGGAA | GGCGATGCCG | CTTACGAAGA | AAAAATCTTC | 300 |
|------------|------------|------------|------------|------------|------------|-----|
| GAACTGGCTA | CCGCATTGGA | CAGATACATC | CCGACTCCCG | AGCGTGCCGT | GGACAAACCA | 360 |
| TTCCTGCTGC | CTATCGAAGA | CGTGTTCTCC | ATTTCCGGCC | GCGGTACCGT | AGTCACCGGC | 420 |
| CGTGTAGAGC | GAGGTATCAT | CCACGTTGGT | GACGAGATTG | AAATCGTCGG | TCTGAAAGAA | 480 |
| ACCCAAAAA  | CCACCTGTAC | CGGCGTTGAA | ATGTTCCGCA | AACTGCTGGA | CGAAGGTCAG | 540 |
| GCGGGCGACA | ACGTAGGCGT | ATTGCTGCGC | GGTACCAAAC | GTGAAGACGT | AGAACGCGGT | 600 |
| CAGGTATTGG | CCAAACGGGG | TACTATCACT | CCTCACACCA | AGTTCAAAGC | AGAAGTGTAC | 660 |
| GTATTGAGCA | AAGAAGAGGG | CGGCCCCCAT | ACCCCGTTTT | TCGCCAACTA | CCGTCCCCAA | 720 |
| TTCTACTTCC | GTACCACTGA | CGTAACCGGC | ACGATTACTT | TGGAAAAAGG | TGTGGAAATG | 780 |
| GTAATGCCGG | GTGAGAACGT | AACCATTACT | GTAGAACTGA | TTGCGCCTAT | CGCTATGGAA | 840 |
| GAAGGTCTGC | GCTTTGCGAT | TCGCGAAGGC | GGCCGTACCG | TGGGTGCCGG | С          | 891 |

- (2) INFORMATION FOR SEQ ID NO: 163:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear .
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

2 !

- (A) ORGANISM: Rickettsia prowazekii
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:

AATATGATAA CTGGTGCCGC TCAGATGGAT GGTGCTATAT TAGTAGTTTC TGCTGCTGAT 60 GGTCCTATGC CTCAAACTAG AGAACATATA TTACTGGCAA AACAGGTAGG TGTACCTGCT 120 ATGGTAGTAT TTTTGAATAA AGTAGATATG GTAGATGATC CTGACCTATT AGAATTAGTT 180 GAGATGGAAG TAAGAGAATT ATTATCAAAA TATGGTTTCC CTGGTAATGA AATACCTATT 240 ATTAAAGGTT CTGCACTTCA AGCTTTAGAA GGAAAACCTG AAGGTGAAAA AGCTATTAAT 300 GAGTTAATGA ATGCAGTAGA TACGTATATA CCTCAGCCTA TAGAGCTACA AGATAAACCT 360 TTTTTAATGC CAATAGAGGA TGTATTTTCT ATTTCAGGCA GAGGTACCGT TGTAACTGGT 420 AGAGTGGAGT CAGGCATAAT TAAGGTGGGT GAAGAAATTG AAATAGTAGG TCTAAAAAAT 480 ACGCAAAAAA CGACTTGTAC AGGTGTAGAA ATGTTCAGAA AATTACTTGA TGAAGGACAA 540 TCTGGAGATA ATGTCGGTAT ATTACTACGT GGTACAAAAA GAGAAGAAGT AGAAAGAGGA 600 WO 98/20157 PCT/CA97/00829 -

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CAAGTACTTG CAAAACCTGG GAGCATAAAA CCGCATGATA AATTTGAAGC TGAAGTGTAT 660
GTGCTTAGTA AAGAGGAAGG TGGACGTCAT ACCCCATTTA CTAATGATTA TCGCCCACAG 720
TTCTATTTTA GAACAACAGA TGTTACCGGC ACAATAAAAT TGCCTTCTGA TAAGCAGATG 780
GTTATGCCTG GAGATAATGC TACTTTTCA GTAGAATTAA TTAAGCCGAT TGCTATGCAA 840
GAAGGGTTAA AATTCTCTAT ACGTGAAGGT GGTAGAACAG TAGGAGCCGG T 891

#### (2) INFORMATION FOR SEQ ID NO: 164:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Salmonella typhimurium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:

AACATGATCA CCGGTGCTGC TCAGATGGAC GGCGCGATCC TGGTTGTTGC TGCGACTGAC 60 GGCCCGATGC CGCAGACCCG TGAGCACATC CTGCTGGGTC GTCAGGTAGG CGTTCCGTAC 120 ATCATCGTGT TCCTGAACAA ATGCGACATG GTTGATGACG AAGAGCTGCT GGAACTGGTT 180 GAGATGGAAG TTCGCGAACT GCTGTCTCAG TACGACTTCC CGGGCGACGA CACTCCGATC 240 GTTCGTGGTT CTGCTCTGAA AGCGCTGGAA GGCGACGCAG AGTGGGAAGC GAAAATCATC 300 GAACTGGCTG GCTTCCTGGA TTCTTATATT CCGGAACCAG AGCGTGCGAT TGACAAGCCG TTCCTGCTGC CGATCGAAGA CGTATTCTCC ATCTCCGGTC GTGGTACCGT TGTTACCGGT 420 CGTGTAGAGC GCGGTATCAT CAAAGTGGGC GAAGAAGTTG AAATCGTTGG TATCAAAGAG 480 ACTCAGAAGT CTACCTGTAC TGGCGTTGAA ATGTTCCGCA AACTGCTGGA CGAAGGCCGT 540 GCCGGTGAGA ACGTAGGTGT TCTGCTGCGT GGTATCAAAC GTGAAGAAAT CGAACGTGGT 600 CAGGTACTGG CTAAGCCGGG CACCATCAAG CCGCACACCA AGTTCGAATC TGAAGTGTAC 660 ATTCTGTCCA AAGATGAAGG CGGCCGTCAT ACTCCGTTCT TCAAAGGCTA CCGTCCGCAG 720 TTCTACTTCC GTACTACTGA CGTGACTGGT ACCATCGAAC TGCCGGAAGG CGTAGAGATG 780 GTAATGCCGG GCGACAACAT CAAAATGGTT GTTACCCTGA TCCACCCGAT CGCGATGGAC 840 GACGGTCTGC GTTTCGCAAT CCGTGAAGGC GGCCGTACCG TTGGCGCGGG C 891

(2) INFORMATION FOR SEQ ID NO: 165:

- (A) LENGTH: 881 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Shewanella putida
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:

ATGATCACTG GTGCTGCACA GATGGACGGC GCGATTCTGG TAGTCGCTTC AACAGACGGT 60 CCAATGCCAC AGACTCGTGA GCACATCCTG CTTTCTCGTC AGGTTGGCGT ACCATTCATC 120 ATCGTATTCA TGAACAAATG TGACATGGTA GATGACGAAG AGCTGTTAGA GCTAGTTGAG 180 ATGGAAGTGC GTGAACTGTT ATCAGAATAC GATTTCCCAG GTGATGACTT ACCGGTAATC 240 CAAGGTTCAG CTCTGAAAGC GCTAGAAGGC GAGCCAGAGT GGGAAGCAAA AATCCTTGAA 300 TTAGCAGCGG CGCTGGATTC TTACATTCCA GAACCACAAC GTGACATCGA TAAGCCGTTC 360 CTACTGCCAA TCGAAGACGT ATTCTCAATT TCAGGCCGTG GTACAGTAGT AACAGGTCGT 420 GTTGAGCGTG GTATTGTACG CGTAGGCGAC GAAGTTGAAA TCGTTGGTGT ACGTGCGACA 480 540 ACTAAGACAA CGTGTACTGG TGTAGAAATG TTCCGTAAAC TGCTTGACGA AGGTCGTGCA 600 GGTGAGAACT GTGGTATTTT GTTACGTGGT ACTAAGCGTG ATGACGTAGA ACGTGGTCAA 660 GTATTAGCGA AGCCAGGTTC AATCAACCCA CACACTACTT TTGAATCAGA AGTTTACGTA 720 CTGTCAAAAG AAGAAGGTGG TCGTCACACG CCATTCTTCA AAGGCTACCG TCCACAGTTC TACTTCCGTA CAACTGACGT AACCGGTACT ATCGAACTGC CAGAAGGCGT AGAGATGGTA 780 ATGCCAGGCG ATAACATCAA GATGGTAGTG ACACTGATTT GCCCAATCGC GATGGACGAA 840 881 GGTTTACGCT TCGCAATCCG TGAAGGCGGT CGTACAGTGG T

- (2) INFORMATION FOR SEQ ID NO: 166:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 897 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Stigmatella aurantiaca

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| (xi) S     | EQUENCE DES  | CRIPTION: SI | EQ ID NO: 16 | 56:        |            |     |
|------------|--------------|--------------|--------------|------------|------------|-----|
| AACATGATCA | CGGGCGCGGC   | GCAGATGGAC   | GGAGCGATTC   | TGGTGGTGTC | CGCGGCCGAC | 60  |
| GGCCCGATGC | CCCAGACGCG   | TGAGCACATC   | CTGCTGGCCA   | GGCAGGTGGG | CGTGCCCTAC | 120 |
| ATCGTCGTCT | TCCTGAACAA   | GGTGGACATG   | CTGGACGATC   | CGGAGCTGCG | CGAGCTGGTG | 180 |
| GAGATGGAGG | TGCGCGACCT   | GCTCAAGAAG   | TACGAGTTCC   | CGGGCGACAG | CATCCCCATC | 240 |
| ATCCCTGGCA | GCGCGCTCAA   | GGCGCTGGAG   | GGAGACACCA   | GCGACATCGG | CGAGGGAGCG | 300 |
| ATCCTGAAGC | TGATGGCGGC   | GGTGGACGAG   | TACATCCCGA   | CGCCGCAGCG | TGCGACGGAC | 360 |
| AAGCCGTTCC | TGATGCCGGT   | GGAAGACGTG   | TTCTCCATCG   | CAGGCCGAGG | AACGGTGGCG | 420 |
| ACGGGCCGAG | TGGAGCGCGG   | CAAGATCAAG   | GTGGGCGAGG   | AAGTGGAGAT | CGTGGGGATC | 480 |
| CGTCCGACGC | AGAAGACGGT   | CATCACGGGG   | GTGGAGATGT   | TCCGCAAGCT | GCTGGACGAG | 540 |
| GGCATGGCGG | GAGACAACAT   | CGGAGCGCTG   | CTGCGAGGCC   | TGAAGCGCGA | GGACCTGGAG | 600 |
| CGTGGGCAGG | TGCTGGCGAA   | CTGGGGGAGC   | ATCAACCCGC   | ACACGAAGTT | CAAGGCGCAG | 660 |
| GTGTACGTGC | TGTCGAAGGA   | AGAGGGAGGG   | CGGCACACGC   | CGTTCTTCAA | GGGATACCGG | 720 |
| CCGCAGTTCT | ACTTCCGGAC   | GACGGACGTG   | ACCGGAACGG   | TGAAGCTGCC | GGACAACGTG | 780 |
| GAGATGGTGA | TGCCGGGAGA   | CAACATCGCC   | ATCGAGGTGG   | AGCTCATTAC | TCCGGTCGCC | 840 |
| ATGGAGAAGG | AGCTGCCGTT   | CGCCATCCGT   | GAGGGTGGCC   | GCACGGTGGG | CGCCGGC    | 897 |
| (2) INFORM | ATION FOR SI | EQ ID NO: 16 | 57:          |            |            |     |

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 894 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus pyogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:

AACATGATCA CTGGTGCCGC TCAAATGGAC GGAGCTATCC TTGTAGTTGC TTCAACTGAT 60
GGACCAATGC CACAAACTCG TGAGCACATC CTTCTTTCAC GTCAGGTTGG TGTTAAACAC 120
CTTATCGTGT TCATGAACAA AGTTGACCTT GTTGATGACG AAGAGTTGCT TGAATTAGTT 180
GAGATGGAAA TTCGTGACCT TCTTTCAGAA TACGATTTCC CAGGTGATGA CCTTCCAGTT 240
ATCCAAGGTT CAGCTCTTAA AGCTCTTGAA GGCGACACTA AATTTGAAGA CATCATCATG 300

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GAATTGATGG ATACTGTTGA TTCATACATT CCAGAACCAG AACGCGACAC TGACAAACCA 360 TTGCTTCTTC CAGTCGAAGA CGTATTCTCA ATTACAGGTC GTGGTACAGT TGCTTCAGGA 420 CGTATCGACC GTGGTACTGT TCGTGTCAAC GACGAAATCG AAATCGTTGG TATCAAAGAA 480 GAAACTAAAA AAGCTGTTGT TACTGGTGTT GAAATGTTCC GTAAACAACT TGACGAAGGT 540 CTTGCAGGAG ACAACGTAGG TATCCTTCTT CGTGGTGTTC AACGTGACGA AATCGAACGT 600 GGTCAAGTTA TTGCTAAACC AAGTTCAATC AACCCACACA CTAAATTCAA AGGTGAAGTA 660 TATATCCTTT CTAAAGACGA AGGTGGACGT CACACTCCAT TCTTCAACAA CTACCGTCCA 720 CAATTCTACT TCCGTACAAC TGACGTAACA GGTTCAATCG AACTTCCAGC AGGTACAGAA 780 ATGGTTATGC CTGGTGATAA CGTGACAATC AACGTTGAGT TGATCCACCC AATCGCCGTA 840 GAACAAGGTA CTACTTTCTC AATCCGTGAA GGTGGACGTA CTGTTGGTTC AGGT 894

- (2) INFORMATION FOR SEQ ID NO: 168:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 897 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Thiobacillus cuprinus
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:

AACATGATCA CCGGTGCGGC CCAGATGGAC GGCGCCATCC TGGTCGTGTC CGCCGCCGAC GGCCCCATGC CCCAAACCCG CGAGCACATC CTGCTGGCGC GTCAGGTGGG CGTGCCCTAC 120 ATCATCGTGT TCCTCAACAA GTGCGACATG GTCGACGACG CCGAGCTGCT CGAACTCGTC 180 240 GAGATGGAAG TGCGCGAGCT GCTGTCCAAG TACGACTTCC CCGGTGACGA CACCCCCATC 300 ATCAAGGGCT CGGCCAAGCT GGCCCTCGAA GGCGACAAGG GCGAACTGGG CGAAGGCGCC ATTCTCAAGC TGGCCGAGGC CCTGGACACC TACATCCCCA CGCCCGAGCG GGCCGTCGAC 360 GGCGCGTTCC TCATGCCCGT GGAAGACGTG TTCTCCATCT CCGGGCGCGG CACGGTGGTC 420 ACCGGGCGTG TGGAGCGCGG CATCATCAAG GTCGGCGAGG AAATCGAGAT TGTCGGCCTC 480 AAGCCCACCC TCAAGACCAC CTGCACCGGC GTGGAAATGT TCAGGAAGCT GCTCGACCAG 540 GGCCAGGCCG GCGACAACGT CGGCATCTTG CTGCGCGGCA CCAAGCGCGA GGAAGTCGAG 600 CGCGGCCAGG TGCTGTGCAA ACCCGGCTCG ATCAAGCCCC ACACCCACTT CACCGCCGAG 660

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| GTGTACGTGC | TGAGCAAGGA | CGAGGGCGGC | CGCCACACCC | CCTTCTTCAA | CAACTACCGC | 720  |
|------------|------------|------------|------------|------------|------------|------|
| CCGCAGTTCT | ACTTCCGCAC | CACCGACGTC | ACCGGCGCCA | TCGAACTGCC | CAAGGACAAG | 780  |
| GAAATGGTCA | TGCCCGGCGA | TAATGTGAGC | ATCACCGTCA | AGCTCATCGC | CCCCATCGCC | 840  |
| ATGGAAGAAG | GCCTGCGCTT | CGCCATCCGC | GAAGGCGGCC | GCACCGTCGG | CGCCGGC    | 8,97 |
| 4.2.       |            |            |            |            |            |      |

- (2) INFORMATION FOR SEQ ID NO: 169:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 894 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Treponema pallidum
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:

| AATATGATCA | CGGGTGCTGC | GCAGATGGAC | GGTGGTÄTTC | TCGTCGTGTC | TGCGCCTGAC | 60    |
|------------|------------|------------|------------|------------|------------|-------|
| GGCGTTATGC | CACAGACGAA | GGAGCATCTT | CTGCTCGCCC | GTCAGGTTGG | TGTTCCCTCC | 120   |
| ATCATTGTTT | TTTTGAACAA | GGTTGATTTG | GTTGATGATC | CTGAGTTGCT | AGAGCTGGTG | 180   |
| GAAGAAGAGG | TGCGTGATGC | GCTTGCTGGA | TATGGGTTTT | CGCGTGAGAC | GCCTATCGTC | 240   |
| AAGGGGTCTG | CGTTTAAAGC | TCTGCAGGAT | GGCGCTTCCC | CGGAGGATGC | AGCTTGTATT | 300   |
| GAGGAACTGC | TTGCGGCCAT | GGATTCCTAC | TTTGAAGACC | CAGTGCGTGA | CGACGCAAGA | 360   |
| CCTTTCTTGC | TCTCTATCGA | GGATGTGTAC | ACTATTTCTG | GGCGTGGTAC | CGTTGTCACG | 420   |
| GGGCGCATCG | AATGTGGGGT | AATTAGTCTG | AATGAAGAGG | TCGAGATCGT | CGGGATTAAG | 480   |
| CCCACTAAGA | AAACAGTGGT | TACTGGCATT | GAGATGTTTA | ATAAGTTGCT | TGATCAGGGA | 540   |
| ATTGCAGGTG | ATAACGTGGG | GCTGCTTTTG | CGCGGGGTGG | ATAAAAAAGA | GGTTGAGCGC | 600   |
| GGTCAGGTGC | TTTCTAAGCC | CGGTTCTATT | AAGCCACACA | CCAAGTTTGA | GGCGCAGATC | 660   |
| TACGTGCTCT | CTAAGGAAGA | GGGTGGCCGT | CACAGTCCTT | TTTTTCAAGG | TTATCGTCCG | . 720 |
| CAGTTTTATT | TTAGAACTAC | TGACATTACC | GGTACGATTT | CTCTTCCTGA | AGGGGTAGAC | 780   |
| ATGGTGAAGC | CGGGGGATAA | CACCAAGATT | ATAGGTGAGC | TCATCCACCC | GATAGCTATG | 840   |
| GACAAGGGTC | TGAAGCTTGC | GATTCGTGAA | GGGGGGCGCA | CTATTGCTTC | TGGT       | 894   |
| (0)        |            |            |            |            |            |       |

(2) INFORMATION FOR SEQ ID NO: 170:

(i) SEQUENCE CHARACTERISTICS:

| (A) | LENGTH: 891 base pairs |
|-----|------------------------|
| (B) | TYPE: nucleic acid     |
| (C) | STRANDEDNESS: double   |

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

(A) ORGANISM: Ureaplasma urealyticum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:

AATATGATTA CAGGGGCAGC ACAAATGGAT GGAGCAATTT TAGTTATTGC TGCATCTGAT 60 GGGGTTATGG CTCAAACTAA AGAACATATT TTATTAGCAC GTCAAGTTGG TGTTCCAAAA 120 ATCGTTGTTT TCTTAAACAA ATGTGATTTC ATGACAGATC CAGATATGCA AGATCTTGTT 180 GAAATGGAAG TTCGTGAATT ATTATCTAAA TATGGATTTG ATGGCGATAA CACACCAGTT 240 ATTCGTGGTT CAGGTCTTAA GGCTTTAGAA GGAGATCCAG TTTGAGAAGC AAAAATTGAT 300 GAATTAATGG ACGCAGTTGA TTCATGAATT CCATTACCAG AACGTAGTAC TGACAAACCA 360 TTCTTATTAG CAATTGAAGA TGTATTCACA ATTTCAGGAC GTGGTACAGT AGTAACTGGA 420 CGTGTTGAAC GTGGTGTATT AAAAGTTAAT GATGAGGTTG AAATTGTTGG TCTAAAAGAC 480 ACTCAAAAAA CTGTTGTTAC AGGAATTGAA ATGTTTAGAA AATCATTAGA TCAAGCTGAA 540 GCTGGTGATA ATGCTGGTAT TTTATTACGT GGTATTAAAA AAGAAGATGT TGAACGTGGT 600 CAAGTACTTG TAAAACCAGG ATCAATTAAA CCTCACCGTA CTTTTACTGC TAAAGTTTAT 660 ATTCTTAAAA AAGAAGAAGG TGGACGTCAT ACACCTATTG TTTCAGGATA CCGTCCACAA 720 TTCTATTTTA GAACAACAGA TGTAACAGGT GCTATTTCAT TACCTGCTGG TGTTGATTTG 780 GTTATGCCAG GTGATGACGT TGAAATGACT GTAGAATTAA TTGCTCCAGT TGCGATTGAA 840 GATGGATCTA AATTCTCAAT CCGTGAAGGT GGTAAAACTG TAGGTCATGG T 891

- (2) INFORMATION FOR SEQ ID NO: 171:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 909 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Wolinella succinogenes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

| AACATGATTA | CAGGTGCTGC | TCAAATGGAT | GGCGCGATTC | TTGTTGTTTC | TGCGGCGGAT | 60                 |
|------------|------------|------------|------------|------------|------------|--------------------|
| GGCCCCATGC | CCCAAACTAG | GGAGCACATT | CTTCTTTCTC | GACAAGTAGG | CGTTCCTTAC | 120                |
| ATCGTGGTTT | TCTTGAACAA | AGAAGATATG | GTTGATGACG | CTGAGCTTCT | TGAGCTTGTT | 180                |
| GAAATGGAAG | TTAGAGAACT | TCTTAGCAAC | TACGACTTCC | CTGGAGATGA | CACTCCTATC | 240                |
| GTTGCAGGTT | CCGCTCTTAA | AGCTCTTGAA | GAGGCTAACG | ACCAGGAAAA | TGTTGGCGAG | 300                |
| TGGGGCGAGA | AAGTATTGAA | GCTTATGGCT | GAGGTTGACC | GATATATTCC | TACGCCTGAG | 360                |
| CGAGATGTGG | ATAAGCCTTT | CCTTATGCCT | GTTGAAGACG | TATTCTCCAT | CGCGGGTCGT | . 420              |
| GGAACCGTTG | TGACAGGAAG | AATTGAAAGA | GGCGTGGTTA | AAGTCGGTGA | CGAAGTAGAA | 480                |
| ATCGTTGGTA | TCCGAAACAC | ACAAAAAACA | ACCGTAACTG | GCGTTGAGAT | GTTCCGAAAA | 540                |
| GAGCTCGACA | AGGGTGAGGC | GGGTGACAAC | GTTGGTGTTC | TTTTGAGAGG | CACCAAGAAA | 600                |
| GAAGATGTTG | AGAGAGGTAT | GGTTCTTTGT | AAAATAGGTT | CTATCACTCC | TCACACTAAC | 660                |
| TTTGAAGGTG | AAGTTTACGT | TCTTTCCAAA | GAGGAAGGCG | GACGACACAC | TCCATTCTTC | 720                |
| AATGGATACC | GACCTCAGTT | CTATGTTAGA | ACTACAGACG | TTACCGGTTC | TATCTCTCTT | 780                |
| CCTGAGGGCG | TAGAGATGGT | TATGCCTGGT | GACAACGTTA | AGATCAATGT | TGAGCTTATC | 840                |
| GCTCCTGTAG | CCCTCGAAGA | GGGAACACGA | TTCGCGATCC | GTGAAGGTGG | TCGAACCGTT | <sup>7,‡</sup> 900 |
| GGTGCGGGT  |            | ·          |            |            |            | 909                |

#### (2) INFORMATION FOR SEQ ID NO: 172:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:6
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:18
  - (D) OTHER INFORMATION:/note= "n = inosine"

GAATAATTCC ATATCCTCCG

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|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:   |    |
|------|--|----|
| TART | TCNGTRA ANGCYTCNAC RCACAT  | 26 |
| (2)  | INFORMATION FOR SEQ ID NO: 173:  |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 21 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:   |    |
| TCTI | TTAGCAG AACAGGATGA A   | 21 |
| (2)  | INFORMATION FOR SEQ ID NO: 174:  |    |
|      | <ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 20 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> |    |
|      | (ii) MOLECULE TYPE: DNA (genomic)  |    |
|      | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:   |    |

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#### **CLAIMS**

#### What is claimed is:

- 1. A method using probes and/or amplification primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids:
- from a bacterial antibiotic resistance gene selected from the group consisting of  $bla_{tem}$ ,  $bla_{shv}$ ,  $bla_{oxa}$ ,
- from specific bacterial and fungal species selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species,

in any sample suspected of containing said bacterial and/or fungal nucleic acids,

wherein each of said nucleic acid or variant or part thereof comprises a selected target region hybridizable with said probes or primers;

said method comprising the following steps: contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said specific bacterial and/or fungal species and bacterial antibiotic resistance genes.

- 2. A method according to claim 1, which further makes use of probes and/or primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids from any bacterium or fungus.
- 3. The method of claim 1, which is performed directly from a test sample.
- 4. The method of claim 1, which is performed directly from a test sample consisting of a bacterial and/or fungal culture or suspension.
  - 5. The method of claim 1, wherein said nucleic acids are all detected under uniform hybridization or amplification conditions.
- 6. The method of claim 1, wherein said nucleic acids are amplified by a method selected from the group consisting of:
  - a) polymerase chain reaction (PCR),
  - b) ligase chain reaction (LCR),
  - c) nucleic acid sequence-based amplification (NASBA),

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- d) self-sustained sequence replication (3SR),
- e) strand displacement amplification (SDA),
- f) branched DNA signal amplification (bDNA),
- g) transcription-mediated amplification (TMA),
- h) cycling probe technology (CPT),
- i) nested PCR, and
- j) multiplex PCR.
- 7. The method of claim 6, wherein said nucleic acids are amplified by PCR.
- 8. The method of claim 7, wherein the PCR protocol achieves within one hour under uniform amplification conditions the determination of the presence of said nucleic acids by performing for each amplification cycle an annealing step of thirty seconds at 45-55°C and a denaturation step of only one second at 95°C without any time specifically allowed to an elongation step.
  - 9. A method for the detection, identification and/or quantification of a microorganism selected from the group consisting of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species, *Streptococcus* species and *Candida* species, directly from a test sample or from bacterial and/or fungal cultures, which comprises the following steps:
    - a) depositing and fixing on an inert support or leaving in solution the said microorganism DNA of the sample or of a substantially homogeneous population of said microorganism isolated from this sample, or
  - inoculating said sample or said substantially homogeneous population of microorganism isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or said isolated microorganism to release the said microorganism DNA,

said microorganism DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleic acid which nucleotide sequence is selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Enterococcus faecium*, *Listeria* 

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monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, respectively, under conditions such that the nucleic acid of said probe can selectively hybridize with said microorganism DNA, whereby a hybridization complex is formed; and

- c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of said microorganism, in said test sample.
- 10. A method for detecting the presence and/or amount of a microorganism selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, in a test sample which comprises the following steps:
- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said microorganism DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, respectively with regard to said microorganism, a sequence complementary thereof, and a variant thereof;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
  - c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of said microorganisms, in said test sample.
  - 11. The method of claim 10, wherein said pair of primers is defined in SEQ ID NOs: 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, 15 and 16, 17 to 20, 21 and 22, respectively, for each of *Enterococcus faecium*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Candida albicans*, *Enterococcus* species, *Neisseria* species, *Staphylococcus* species and *Streptococcus* species.

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- 12. A method for detecting the presence and/or amount of any bacterium directly from a test sample or a bacterial culture, which comprises the following steps:
- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

inoculating said sample or said substantially homogeneous population of bacteria isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being made in a substantially single-stranded form;

- b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleic acid which nucleotide sequence is selected from the group consisting of SEQ ID NOs: 118, 119, 125 to 171, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of any bacterial species, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed; and
  - c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of any bacterium in said test sample.
  - 13. A method for detecting the presence and/or amount of any bacterium in a test sample which comprises the following steps:
  - a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of any bacterial DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NO: 118, 119, 125 to 171, a sequence complementary thereof, and a variant thereof;
  - b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of any bacterium in said test sample.

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- 14. The method of claim 13, wherein said pair of primers is defined in SEQ ID NOs: 23 and 24.
- 15. A method for obtaining *tuf* sequences from any bacterium directly from a test sample or a bacterial culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequences defined in SEQ ID NOs: 107 and 108, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial *tuf* gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
  - c) detecting the presence and/or amount of said amplified target sequence; and
- d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.
- 16. A method for detecting the presence and/or amount of any fungus directly from a test sample or a fungal culture, which comprises the following steps:
  - a) depositing and fixing on an inert support or leaving in solution the fungal DNA of the sample or of a substantially homogeneous population of fungi isolated from this sample, or

inoculating said sample or said substantially homogeneous population of fungi isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated fungi to release the fungal DNA,

said fungal DNA being made in a substantially single-stranded form;

- b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence selected from the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of any fungus, under conditions such that the nucleic acid of said probe can selectively hybridize with said fungal DNA, whereby a hybridization complex is formed; and
  - c) detecting the presence of said hybridization complex on said inert support or SUBSTITUTE SHEET (RULE 26)

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in said solution as an indication of the presence and/or amount of any fungus in said test sample.

- 17. A method for detecting the presence and/or amount of any fungus in a test sample which comprises the following steps:
- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of any fungal DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, and a variant thereof;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of any fungus in said test sample.
- 18. A method for obtaining *tuf* sequences from any fungus directly from a test sample or a fungal culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 109 and 172, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said fungal *tuf* gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
  - c) detecting the presence and/or amount of said amplified target sequence; and
- d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.
- 19. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected

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from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC, directly from a test sample or a bacterial culture, which comprises the following steps:

a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

inoculating said sample or said substantially homogeneous population of bacteria isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence having at least 12 nucleotide in length is selected from the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114 115, 116, 117, a sequence complementary thereof, and a variant thereof, which specifically hybridizes with said bacterial antibiotic resistance gene, respectively; and

- c) detecting the presence of a hybridization complex as an indication of a bacterial resistance mediated by said one of said bacterial antibiotic resistance genes.
- 20. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected from the group consisting of bla<sub>oxe</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC, directly from a test sample or a bacterial culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing at least one pair of primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114, 115, 116, 117, respectively with regard to said bacterial antibiotic resistance gene, a sequence complementary thereof, and a variant thereof;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.

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- 21. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance gene selected from the group consisting of  $bla_{tem}$ ,  $bla_{rob}$ ,  $bla_{coa}$ ,  $bla_{coa$
- a) treating said sample with an aqueous solution containing at least one pair of primers having a sequence selected in the group consisting of SEQ ID NOs: 37 to 40, 41 to 44, 45 to 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 to 60, 61 to 64, 65 and 66, 173 and 174, 67 to 70, 71 to 74, 75 and 76, 77 to 80, 81 and 82, 83 to 86, 87 and 88, 89 and 90, 91 and 92, 93 and 94, 95 and 96, 97 and 98, 99 to 102, 103 to 106, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, a variant thereof, and mixtures thereof, one of said primers of said pair being capable of hybridizing selectively with one of the two complementary strands of its respective bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers of said pairs being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.
- 22. A nucleic acid having the nucleotide sequence of any one of SEQ ID NOs: 26 to 36, 110 to 171, a part thereof, a sequence complementary thereof, and variant thereof which, when in single-stranded form, ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.
  - 23. An oligonucleotide having the nucleotide sequence of any one of SEQ ID NOs: 1 to 25, 37 to 109, 172 to 174, a part thereof, a sequence complementary thereof, and variant thereof, which ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.
  - 24. A recombinant plasmid comprising a nucleic acid as defined in claim 22.
  - 25. A recombinant host which has been transformed by a recombinant plasmid according to claim 24.
- 35 26. A recombinant host according to claim 25 wherein said host is Escherichia coli.

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27. A diagnostic kit for the detection and/or quantification of the nucleic acids of any SUBSTITUTE SHEET (RULE 26)

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combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, comprising any suitable combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.

- 28. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.
  - 29. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species and Streptococcus species, comprising any suitable combination of primers selected from the group consisting of SEQ ID NOs: 1 to 22, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.
  - 30. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC, comprising any suitable combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof.
  - 31. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof.

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32. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>tem</sub>, bla<sub>rob</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, bla<sub>oxa</sub>, aacC1, aacC2, aacC3, aac6'-lla, aacA4, aad(6'), vanA, vanB, vanC, msrA, satA, aac(6')-aph(2"), vat, vga, ermA, ermB, ermC, mecA, int and sul, comprising any suitable combination of primers selected from the group consisting of SEQ ID NOs: 37 to 106, 173 and 174, a part thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.

A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.

- 34. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.
- 35. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium, comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.
- 36. A diagnostic kit, as defined in claim 27, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 25 37. A diagnostic kit, as defined in claim 28, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 38. A diagnostic kit, as defined in claim 29, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.
- 35 39. A diagnostic kit, as defined in claim 27, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from

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the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC.

- 5 40. A diagnostic kit, as defined in claim 28, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC.
  - 41. A diagnostic kit, as defined in claim 29, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 37 to 106, 173 and 174, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of  $bla_{tem}$ ,  $bla_{rob}$ ,  $bla_{shv}$ ,  $bla_{oxa}$ ,
- 42. A diagnostic kit, as defined in claim 30, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 43. A diagnostic kit, as defined in claim 31, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
  - 44. A diagnostic kit, as defined in claim 32, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.
  - 45. A diagnostic kit, as defined in claim 39, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic

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acids of any bacterium and/or fungus.

- 46. A diagnostic kit, as defined in claim 40, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 47. A diagnostic kit, as defined in claim 41, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.

Inter. onal Application No PCT/CA 97/00829

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| Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)  |
|--|
| This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:   |
| 1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:   |
| Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically: |
| 3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).  |
| Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)  |
| This International Searching Authority found multiple inventions in this international application, as follows:  |
| see additional sheet   |
|  |
| 1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.  |
| 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.  |
| 3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:                    |
| 4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:        |
| Remark on Protest  The additional search fees were accompanied by the applicant's protest.  X No protest accompanied the payment of additional search fees.  |

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-13,19,21-29,32-34,36,41-43,45,46 (partly)

Nucleic acids (SEQ. ID.:1,2,13,14,67-70,51,52, 71-76, 81-86,131-134, 173,174) specific for Enterococcus spp., methods using them, and plasmids, hosts and kits containing them

2. Claims: 1-9,11-13,22-26,29,33,34,36,37,42,43,45,46 (partly)

Nucleic acids (SEQ ID.: 3,4, 136-139) specific for Listeria monocytogenes, methods using them, and plasmids, hosts and kits containing them

3. Claims: 1-9,11-13,22-26,29,33,34,36,37,42,43,45,46 (partly)

Nucleic acids (SEQ. ID.: 5,6,15,16,162) specific for Neisseria spp., methods using them, and plasmids, hosts and kits containing them

4. Claims: 1-9,11-13,21-26,32-34,36,37,42,43,45,46 (partly)

Nucleic acids (SEQ ID.: 7,8,17-20,77-80,89-98, 140-143) specific for Staphylococcus spp., methods using them, and plasmids, hosts and kits containing them

5. Claims: 1-9,11-13,22-26,29,33,34,36,37,42,43,45,46 (partly)

Nucleic acids (SEQ. ID.: 9,10,21,22,144-146,167) specific for Streptococcus spp., methods using them, and plasmids, hosts and kits containing them

6. Claims: 1-12,22-29,33,34,36,37,42,43,45,46 (partly), 16, 17 (complete)

Nucleic acids (SEQ ID.: 11,12,120-124) specific for Candida albicans, methods using them, and plasmids, hosts and kits containing them

7. Claims: 14,19,20,30,31,35,38,39,40,44,47 (complete), 23, 32 (partly)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Nucleic acids (SEQ ID.: 23-24, 99-106, 110-117, 118,119, 125-130, 135, 147-161, 163-166, 168-171) specific for universal detection of bacteria and fungus species, methods using them, and plasmids, hosts and kits containing them

8. Claims: 15,18 (complete)

Methods for obtaining tuf sequences by using SEQ ID.: 107,108,109 and 172

Information on patent family members

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